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<p>(21) International Application Number: PCT/US99/08524</p> <p>(22) International Filing Date: 16 April 1999 (16.04.99)</p> <p>(30) Priority Data:</p> <table border="0"><tr><td>09/062,013</td><td>17 April 1998 (17.04.98)</td><td>US</td></tr><tr><td>60/085,295</td><td>15 May 1998 (13.05.98)</td><td>US</td></tr><tr><td>60/098,900</td><td>2 September 1998 (02.09.98)</td><td>US</td></tr></table> <p>(71) Applicant: HESKA CORPORATION (US), 1613 Prospect Parkway, Fort Collins, CO 80525 (US)</p> <p>(72) Inventors: McCALL, Catherine Anne, 709 Pleasant Street, Boulder, CO 80502 (US); HUNTER, Shirley Wu, 2325 Tanglewood Drive, Fort Collins, CO 80525 (US); WEBER, Eric R., 2625 Silver Creek Drive, Fort Collins, CO 80525 (US).</p> <p>(74) Agents: CONNELL, Gary J. et al., Sheridan Ross P.C., Suite 3500, 1700 Lincoln Street, Denver, CO 80203-4501 (US).</p>		09/062,013	17 April 1998 (17.04.98)	US	60/085,295	15 May 1998 (13.05.98)	US	60/098,900	2 September 1998 (02.09.98)	US	<p>(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).</p> <p><b>Published</b> <i>Without international search report and to be republished upon receipt of that report.</i></p>
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(54) Title: *DERMATOPHAGOIDES* NUCLEIC ACID MOLECULES, PROTEINS AND USES THEREOF

## (57) Abstract

The present invention relates to high molecular weight *Dermatophagoides* proteins, nucleic acid molecules encoding such proteins, and therapeutic and diagnostic reagents derived from such proteins.

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# NOVEL DERMATOPHAGOIDES NUCLEIC ACID MOLECULES, PROTEINS AND USES THEREOF

## FIELD OF THE INVENTION

The present invention relates to high molecular weight *Dermatophagoides* proteins, nucleic acid molecules and therapeutic and diagnostic reagents derived from such proteins.

## BACKGROUND OF THE INVENTION

Immunoglobulin E (IgE) mediated allergic symptoms afflict many animals. IgE antibody production in an animal can induce pathogenic IgE responses including, for example, atopic disease, asthma and rhinitis. Allergens are proteins or peptides characterized by their ability to induce a pathogenic IgE response in susceptible individuals.

House dust mite (e.g., *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus*; *Der f* and *Der p*, respectively) allergens are major causative agents associated with IgE-mediated pathogenesis. Previous investigators have identified two major groups of dust mite allergens in humans, group I (*Der f* I and *Der p* I, Mr 25,000) and group 2 (*Der f* II and *Der p* II, Mr 14,000); reviewed in Chapman, et al., *Allergy*, vol. 52, pp.37-379, 1997. Prior investigators have disclosed nucleotide and/or amino acid sequences for: *Der f* I, *Der f* II, *Der p* I and *Der p* II, U.S. Patent No. 5,552,142, to Thomas et al., issued September 3, 1996, U.S. Patent No. 5,460,977, to Ando et al., issued October 24, 1995, PCT Patent Publication No. WO 95/28424, by Chen et al., published October 26, 1995, U.S. Patent No. 5,433,948, to Thomas et al., issued July 18, 1995, PCT Patent Publication No. WO 93/08279, by Garmen et al., published March 4, 1993, or Chapman, *ibid.*: *Der p* III, PCT Patent Publication No. WO 95/15976, by Thomas et al., published June 15, 1995; *Der p* VII, PCT Patent Publication No. WO 94/20614, by Thomas et al., published September 15, 1994; a 40-kilodalton (kd) *Der f* allergen, U.S. Patent No. 5,405,758, to Oka et al., issued April 11, 1995, U.S. Patent No.

and *Der p* I, *Immunopath.*, vol. 52, pp. 147-157, 1996; and a 98-kd *Der f* paramyosin-like allergen, Tsai et al., *J. Allergy Clin. Immunol.*, vol. 102, pp. 295-303,

1998. None of these published sequences indicates, suggests or predicts any of the mite allergic nucleic acid molecules or proteins of the present invention, nor the relevance of such proteins as being immunoreactive with IgE antibodies in canine, feline, or human sera.

- 5           Products and processes of the present invention are needed in the art that provide specific detection and treatment of mite allergy.

#### SUMMARY OF THE INVENTION

- The present invention relates to novel proteins having molecular weights of about 60 kilodaltons (kd or kD), 70 kD, or from about 98 kD to about 109 kD. Such
- 10   proteins include at least one epitope of a protein allergen of a mite of the genus *Dermatophagoides* and are designated herein as *Der* HMW-map proteins. Preferred proteins are *Dermatophagoides farinae* or *Dermatophagoides pteronyssius* proteins. The present invention also provides proteins that are fragments or peptides of full-length or mature proteins, as well as antibodies, mimetopes or muteins of any of such proteins.
- 15   The present invention also provides nucleic acid molecules encoding any of such proteins, as well as complements thereof. The present invention also includes methods to obtain such proteins, nucleic acid molecules, antibodies, mimetopes or muteins, as well as methods to use such compounds in diagnostic or therapeutic applications. The present invention also relates to reagents comprising non-proteinaceous epitopes that
- 20   bind to IgE in mite-allergic dogs and/or cats as well as to antibodies raised against such epitopes. The present invention also relates to therapeutic compositions or assay kits comprising such non-proteinaceous epitopes, as well as to methods to identify and/or desensitize an animal susceptible to an allergic response to a mite, comprising the use of non-proteinaceous epitopes of the present invention.
- 25           One embodiment of the present invention is at least one of the following isolated nucleic acid molecules: (a) a nucleic acid molecule comprising at least about 150 nucleotides, wherein such a nucleic acid molecule hybridizes, in a solution comprising 1X SSC and 0% formamide, at a temperature of about 50°C, to a nucleic acid molecule comprising at least one of the following nucleic acid sequences: SEQ ID NO:14, SEQ ID
- 30   NO:16, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:40, SEQ ID



NO:42, SEQ ID NO:43, SEQ ID NO:45, and a nucleic acid sequence encoding a protein comprising the amino acid sequence of SEQ ID NO:33 and a complement thereof; and  
 (b) a nucleic acid molecule comprising a fragment of any of the nucleic acid molecules of (a) wherein the fragment comprises at least about 15 nucleotides. The present  
 5 invention also includes recombinant molecules, recombinant viruses and recombinant cells comprising such nucleic acid sequences as well as methods to produce them.

Another embodiment of the present invention is an isolated protein encoded by at least one of the following nucleic acid molecules: (a) a nucleic acid molecule comprising at least about 150 nucleotides, wherein such a nucleic acid molecule  
 10 hybridizes, in a solution comprising 1X SSC and 0% formamide, at a temperature of about 50°C, to a nucleic acid molecule comprising at least one of the following nucleic acid sequences: SEQ ID NO:16, SEQ ID NO:19, SEQ ID NO:22, SEQ ID NO:36, SEQ ID NO:39, SEQ ID NO:42, SEQ ID NO:45, and a complement of a nucleic acid sequence encoding a protein comprising the amino acid sequence SEQ ID NO:33; and  
 15 (b) a nucleic acid molecule comprising a fragment of any of the nucleic acid molecules of (a), wherein the fragment comprises at least about 15 nucleotides. An isolated protein of the present invention can also be encoded by a nucleic acid molecule that hybridizes under stringent hybridization conditions with the complement of a nucleic acid molecule that encodes a protein having at least one of the following amino acid sequences: SEQ  
 20 ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:18, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:38, SEQ ID NO:41, and SEQ ID  
 25 NO:44. The present invention also includes an antibody that selectively binds to a protein of the present invention as well as methods to produce and use such proteins or antibodies.

3. The present invention also includes the following desensitizing compounds: (a) an isolated nucleic acid molecule of the present invention; (b) an isolated mite allergenic protein of the present invention; (c) a mimotope

of such a mite allergenic protein; (d) a mutein of such a mite allergenic protein; (e) an antibody to such a mite allergenic protein; and (f) an inhibitor of binding of such a mite allergenic protein to IgE. Also included is a method to desensitize a host animal to an allergic response to a mite. Such a method includes the step of administering to the  
5 animal a therapeutic composition of the present invention.

One embodiment of the present invention is an assay kit for testing if an animal is susceptible to or has an allergic response to a mite. Such a kit includes an isolated protein of the present invention and a means for determining if the animal is susceptible to or has that allergic response. Such a means includes use of such a protein to identify  
10 animals susceptible to or having allergic responses to mites. The present invention also includes a method to identify an animal susceptible to or having an allergic response to a mite. Such a method includes the steps of: (a) contacting an isolated protein of the present invention with antibodies of an animal; and (b) determining immunocomplex formation between the protein and the antibodies, wherein formation of the  
15 immunocomplex indicates that the animal is susceptible to or has such an allergic response.

The present invention includes a reagent that comprises a non-proteinaceous epitope having at least one of the following identifying characteristics: (a) the epitope is resistant to  $\beta$ -elimination of peptides; (b) the epitope is resistant to Proteinase-K  
20 digestion; and (c) the epitope is reactive to a test designed to detect glycosylated proteins. Such an epitope binds to at least one of the following antibodies: canine IgE from dogs allergic to mites and feline IgE from cats allergic to mites. Also included is an isolated antibody that selectively binds such a non-proteinaceous epitope as well as derivatives of such an epitope.

25 The present invention also relates to therapeutic compositions and assay kits comprising a non-proteinaceous epitope of the present invention, as well as methods to identify and/or desensitize an animal susceptible to an allergic response to a mite, comprising the use of a non-proteinaceous epitope of the present invention.

#### BRIEF DESCRIPTION OF THE FIGURES

30 Fig. 1 illustrates high molecular weight *Der f* proteins resolved by 12% Tris-Glycine SDS-PAGE.

Fig. 2 illustrates an about 60 kD *Der f* protein resolved by 14% Tris-Glycine SDS-PAGE.

#### DETAILED DESCRIPTION OF THE INVENTION

The present invention provides for isolated proteins having molecular weights  
5 ranging from about 60 kilodaltons (kD) to about 109 kD, that include at least one  
epitope of a protein allergen of a mite of the genus *Dermatophagoides*, in particular a  
mite of the species *Dermatophagoides farinae* and/or *Dermatophagoides pteronyssius*.  
Such proteins are referred to herein as *Der* HMW-map proteins. The present invention  
further includes methods to isolate and identify nucleic acid molecules encoding  
10 *Der*HMW-map proteins, antibodies directed against *Der* HMW-map proteins and  
inhibitors of *Der* HMW-map protein activity. As used herein, the term isolated *Der*  
HMW-map proteins refers to *Der* HMW-map proteins derived from *Dermatophagoides*,  
and more preferably from *Dermatophagoides farinae* and/or *Dermatophagoides*  
*pteronyssius* and, as such, can be obtained from its natural source or can be produced  
15 using, for example, recombinant nucleic acid technology or chemical synthesis. Also  
included in the present invention is the use of this protein and antibodies in a method to  
detect immunoglobulin that specifically binds to *Der* HMW-map proteins, to treat  
pathogenesis against mite allergens, and in other applications, such as those disclosed  
below. The products and processes of the present invention are advantageous because  
20 they enable the detection of anti-*Der* HMW-map antibodies in fluids of animals and the  
inhibition of IgE or *Der* HMW-map protein activity associated with disease.

One embodiment of the present invention is an isolated *Dermatophagoides*  
allergenic composition including: (a) a composition produced by a method comprising:  
(1) applying soluble proteins of a *Dermatophagoides* extract to a gel filtration column;  
25 (2) collecting excluded protein from the gel filtration column and applying the excluded  
protein to an anion exchange column; and (3) eluting proteins bound to the anion  
exchange column with about 0.3 M Tris-HCl, pH 8 to obtain the *Dermatophagoides*

30 biological function including binding to IgE, stimulating a B lymphocyte response and  
stimulating a T lymphocyte response. Such *Dermatophagoides* allergenic composition

is also referred to herein as a *Der* HMW-map composition. A suitable gel filtration column includes any gel filtration column capable of excluding proteins having a molecular weight between about 50 kD and about 150 kD. A preferred gel filtration column includes, but is not limited to a Sephacryl S-100 column. A suitable anion exchange column includes any anion exchange column capable of binding to a protein having a pI of less than about pI 6. A preferred anion exchange column includes, but is not limited to a Q-Sepharose column. As used herein, "stimulating a B lymphocyte response" refers to increasing a humoral immune response in an animal that is induced preferentially by a *Der* HMW-map of the present invention and involves the activity of a B lymphocyte in the animal. As used herein, "stimulating a T lymphocyte response" refers to increasing a cellular immune response in an animal that is induced preferentially by a *Der* HMW-map of the present invention and involves the activity of a T lymphocyte in the animal.

One embodiment of the present invention is an isolated protein that includes a *Der* HMW-map protein. It is to be noted that the term "a" or "an" entity refers to one or more of that entity; for example, a protein, a nucleic acid molecule, an antibody, an inhibitor, a compound or a therapeutic composition refers to "one or more" or "at least one" protein, nucleic acid molecule, antibody, inhibitor, compound or therapeutic composition respectively. As such, the terms "a" (or "an"), "one or more" and "at least one" can be used interchangeably herein. It is also to be noted that the terms "comprising", "including", and "having" can be used interchangeably. According to the present invention, an isolated, or biologically pure, protein, is a protein that has been removed from its natural milieu. As such, "isolated" and "biologically pure" do not necessarily reflect the extent to which the protein has been purified. An isolated protein of the present invention can be obtained from its natural source, can be produced using recombinant DNA technology, or can be produced by chemical synthesis.

As used herein, a *Der* HMW-map protein can be a full-length protein or any homolog of such a protein. As used herein, a protein can be a polypeptide or a peptide, as the terms are used by those of skill in the art. Preferably, a *Der* HMW-map protein comprises at least a portion of a *Der* HMW-map protein that comprises at least one epitope recognized by an IgE antibody (i.e., a protein of the present invention binds to an

IgE antibody), an antibody on the surface of a B lymphocyte and or a T cell receptor in the presence of a major histocompatibility complex (MHC) molecule from an animal demonstrating IgE-mediated pathogenesis to a *Der* HMW-map protein.

A peptide of the present invention includes a *Der* HMW-map protein of the present invention that is capable of binding to IgE, desensitizing an animal against mite allergen, stimulating a B lymphocyte response, and/or stimulating a T lymphocyte response. Preferably, a peptide of the present invention comprises a B lymphocyte epitope or a T lymphocyte epitope. A peptide having a B lymphocyte epitope can bind to an antibody. A peptide having a T lymphocyte epitope can bind to a MHC molecule in such a manner that the peptide can stimulate a T lymphocyte through a T cell receptor. According to the present invention, a peptide comprising a B lymphocyte epitope can be from about 4 residues to about 50 residues in length, preferably from about 5 residues to about 20 residues in length. According to the present invention, a peptide comprising a T lymphocyte epitope can be from about 4 residues to about 20 residues in length, preferably from about 8 residues to about 16 residues in length.

A *Der* HMW-map protein of the present invention, including a homolog, can be identified in a straight-forward manner by the protein's ability to induce an allergic response to *Der* HMW-map protein. Examples of *Der* HMW-map protein homologs include *Der* HMW-map protein in which amino acids have been deleted (e.g., a truncated version of the protein, such as a peptide), inserted, inverted, substituted and/or derivatized (e.g., by glycosylation, phosphorylation, acetylation, myristoylation, prenylation, palmitoylation, amidation and/or addition of glycerophosphatidyl inositol) such that the homolog is capable of inducing an allergic response to a natural *Der* HMW-map protein.

*Der* HMW-map protein homologs can be the result of natural allelic variation or natural mutation. *Der* HMW-map protein homologs of the present invention can also be produced using techniques known in the art including, but not limited to, direct

mutagenesis.

One embodiment of the present invention is a *Der* HMW-map gene that includes the nucleic acid sequence SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:43, and SEQ ID NO:45 as well as the complements of any of these nucleic acid sequences. These nucleic acid sequences are further described herein. For example, nucleic acid sequence SEQ ID NO:14 represents the deduced sequence of the coding strand of a cDNA (complementary DNA) denoted herein as *Der* HMW-map gene nucleic acid molecule nDerf98<sub>1752</sub>, the production of which is disclosed in the Examples. Nucleic acid molecule nDerf98<sub>1752</sub> comprises an apparently full-length coding region. The complement of SEQ ID NO:14 (represented herein by SEQ ID NO:16) refers to the nucleic acid sequence of the strand complementary to the strand having SEQ ID NO:14, which can easily be determined by those skilled in the art. Likewise, a nucleic acid sequence complement of any nucleic acid sequence of the present invention refers to the nucleic acid sequence of the nucleic acid strand that is complementary to (i.e., can form a double helix with) the strand for which the sequence is cited. It should be noted that since nucleic acid sequencing technology is not entirely error-free, SEQ ID NO:14 (as well as other nucleic acid and protein sequences presented herein) represents an apparent nucleic acid sequence of the nucleic acid molecule encoding a *Der* HMW-map protein of the present invention.

In another embodiment, a *Der* HMW-map gene or nucleic acid molecule can be an allelic variant that includes a similar but not identical sequence to SEQ ID NO:14 or SEQ ID NO:16, or any other *Der* HMW-map nucleic acid sequence cited herein. For example, an allelic variant of a *Der* HMW-map gene including SEQ ID NO:14 or SEQ ID NO:16, is a gene that occurs at essentially the same locus (or loci) in the genome as the gene including SEQ ID NO:14 and SEQ ID NO:16, but which, due to natural variations caused by, for example, mutation or recombination, has a similar but not identical sequence. Because natural selection typically selects against alterations that affect function, allelic variants (i.e. alleles corresponding to, or of, cited nucleic acid sequences) usually encode proteins having similar activity to that of the protein encoded by the gene to which they are being compared. Allelic variants of genes or nucleic acid

molecules can also comprise alterations in the 5' or 3' untranslated regions of the gene (e.g., in regulatory control regions), or can involve alternative splicing of a nascent transcript, thereby bringing alternative exons into juxtaposition. Allelic variants are well known to those skilled in the art and would be expected to occur naturally within a given  
5 dust mite such as *Dermatophagoides*, since the respective genomes are diploid, and sexual reproduction will result in the reassortment of alleles.

In one embodiment of the present invention, an isolated *Der* HMW-map protein is encoded by a nucleic acid molecule that hybridizes under stringent hybridization conditions to a gene encoding a *Der* HMW-map protein. The minimal size of a *Der*  
10 HMW-map protein of the present invention is a size sufficient to be encoded by a nucleic acid molecule capable of forming a stable hybrid (i.e., hybridizing under stringent hybridization conditions) with the complementary sequence of a nucleic acid molecule encoding the corresponding natural protein. The size of a nucleic acid molecule encoding such a protein is dependent on the nucleic acid composition and the  
15 percent homology between the *Der* HMW-map nucleic acid molecule and the complementary nucleic acid sequence. It can easily be understood that the extent of homology required to form a stable hybrid under stringent conditions can vary depending on whether the homologous sequences are interspersed throughout a given nucleic acid molecule or are clustered (i.e., localized) in distinct regions on a given  
20 nucleic acid molecule.

The minimal size of a nucleic acid molecule capable of forming a stable hybrid with a gene encoding a *Der* HMW-map protein is typically at least about 12 nucleotides to about 15 nucleotides in length if the nucleic acid molecule is GC-rich and at least about 15 to about 17 bases in length if it is AT-rich. The minimal size of a nucleic acid  
25 molecule used to encode a *Der* HMW-map protein homolog of the present invention is from about 12 to about 18 nucleotides in length, preferably about 12 nucleotides, or about 15 nucleotides, or about 18 nucleotides in length. Thus, the minimal size of a *Der*

30 nucleic acid molecule encoding a *Der* HMW-map protein of the present invention because a nucleic acid molecule of the present invention can include a portion of a gene

an entire gene, or multiple genes. The preferred size of a protein encoded by a nucleic acid molecule of the present invention depends on whether a full-length, fusion, multivalent, or functional portion of such a protein is desired. Preferably, the preferred size of a protein encoded by a nucleic acid molecule of the present invention is a portion  
5 of the protein that induces an immune response which is about 30 amino acids, more preferably about 35 amino acids and even more preferably about 44 amino acids in length.

Stringent hybridization conditions are determined based on defined physical properties of the gene to which the nucleic acid molecule is being hybridized, and can be  
10 defined mathematically. Stringent hybridization conditions are those experimental parameters that allow an individual skilled in the art to identify significant similarities between heterologous nucleic acid molecules. These conditions are well known to those skilled in the art. See, for example, Sambrook, *et al.*, 1989, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Labs Press, and Meinkoth, *et al.*, 1984, *Anal.*  
15 *Biochem.* 138, 267-284. As explained in detail in the cited references, the determination of hybridization conditions involves the manipulation of a set of variables including the ionic strength (M, in moles/liter), the hybridization temperature (°C), the concentration of nucleic acid helix destabilizing agents (such as formamide), the average length of the shortest hybrid duplex (n), and the percent G + C composition of the fragment to which  
20 an unknown nucleic acid molecule is being hybridized. For nucleic acid molecules of at least about 150 nucleotides, these variables are inserted into a standard mathematical formula to calculate the melting temperature, or  $T_m$ , of a given nucleic acid molecule. As defined in the formula below,  $T_m$  is the temperature at which two complementary nucleic acid molecule strands will disassociate, assuming 100% complementarity  
25 between the two strands:

$$T_m = 81.5^\circ\text{C} + 16.6 \log M + 0.41(\%G + C) - 500/n - 0.61(\%\text{formamide}).$$

For nucleic acid molecules smaller than about 50 nucleotides, hybrid stability is defined by the dissociation temperature ( $T_d$ ), which is defined as the temperature at which 50% of the duplexes dissociate. For these smaller molecules, the stability at a standard ionic  
30 strength is defined by the following equation:

$$T_d = 4(G + C) + 2(A + T).$$



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A temperature of 5°C below  $T_d$  is used to detect hybridization between perfectly matched molecules.

Also well known to those skilled in the art is how base-pair mismatch, i.e. differences between two nucleic acid molecules being compared, including non-complementarity of bases at a given location, and gaps due to insertion or deletion of one or more bases at a given location on either of the nucleic acid molecules being compared, will affect  $T_m$  or  $T_d$  for nucleic acid molecules of different sizes. For example,  $T_m$  decreases about 1°C for each 1% of mismatched base-pairs for hybrids greater than about 150 bp, and  $T_d$  decreases about 5°C for each mismatched base-pair for hybrids below about 50 bp. Conditions for hybrids between about 50 and about 150 base-pairs can be determined empirically and without undue experimentation using standard laboratory procedures well known to those skilled in the art. These simple procedures allow one skilled in the art to set the hybridization conditions (by altering, for example, the salt concentration, the formamide concentration or the temperature) so that only nucleic acid hybrids with less than a specified % base-pair mismatch will hybridize. Stringent hybridization conditions are commonly understood by those skilled in the art to be those experimental conditions that will allow hybridization between molecules having about 30% or less base-pair mismatch (i.e., about 70% or greater identity). Because one skilled in the art can easily determine whether a given nucleic acid molecule to be tested is less than or greater than about 50 nucleotides, and can therefore choose the appropriate formula for determining hybridization conditions, he or she can determine whether the nucleic acid molecule will hybridize with a given gene under stringent hybridization conditions and similarly whether the nucleic acid molecule will hybridize under conditions designed to allow a desired amount of base pair mismatch.

Hybridization reactions are often carried out by attaching the nucleic acid molecule to be hybridized to a solid support such as a membrane, and then hybridizing with a labeled nucleic acid molecule, typically referred to as a probe, suspended in a

Typically, the actual hybridization reaction is done under non-stringent conditions, i.e., at a lower temperature and/or a higher salt concentration, and then high stringency is

achieved by washing the membrane in a solution with a higher temperature and/or lower salt concentration in order to achieve the desired stringency.

For example, if the skilled artisan wished to identify a nucleic acid molecule that hybridizes under stringent hybridization conditions with a *Dermatophagoides farinae* and/or *Dermatophagoides pteronyssius* nucleic acid molecule of about 150 bp in length, the following conditions could preferably be used. The average G + C content of *Dermatophagoides farinae* and *Dermatophagoides pteronyssius* DNA is about 39%. The unknown nucleic acid molecules would be attached to a support membrane, and the 150 bp probe would be labeled, e.g. with a radioactive tag. The hybridization reaction could be carried out in a solution comprising 2X SSC and 0% formamide, at a temperature of about 37°C (low stringency conditions). Solutions of differing concentrations of SSC can be made by one of skill in the art by diluting a stock solution of 20X SSC (175.3 gram NaCl and about 88.2 gram sodium citrate in 1 liter of water, pH 7) to obtain the desired concentration of SSC. In order to achieve high stringency hybridization, the skilled artisan would calculate the washing conditions required to allow up to 30% base-pair mismatch. For example, in a wash solution comprising 1X SSC and 0% formamide, the  $T_m$  of perfect hybrids would be about 80°C:

$$81.5^{\circ}\text{C} + 16.6 \log (.15\text{M}) + (0.41 \times 39) - (500/150) - (0.61 \times 0) = 80.4^{\circ}\text{C}.$$

Thus, to achieve hybridization with nucleic acid molecules having about 30% base-pair mismatch, hybridization washes would be carried out at a temperature of about 50°C. It is thus within the skill of one in the art to calculate additional hybridization temperatures based on the desired percentage base-pair mismatch, formulae and G/C content disclosed herein. For example, it is appreciated by one skilled in the art that as the nucleic acid molecule to be tested for hybridization against nucleic acid molecules of the present invention having sequences specified herein becomes longer than 150 nucleotides, the  $T_m$  for a hybridization reaction allowing up to 30% base-pair mismatch will not vary significantly from 50°C.

Furthermore, it is known in the art that there are commercially available computer programs for determining the degree of similarity between two nucleic acid sequences. These computer programs include various known methods to determine the percentage identity and the number and length of gaps between hybrid nucleic acid

molecules. Preferred methods to determine the percent identity among amino acid sequences and also among nucleic acid sequences include analysis using one or more of the commercially available computer programs designed to compare and analyze nucleic acid or amino acid sequences. These computer programs include, but are not limited to, 5 GCG<sup>TM</sup> (available from Genetics Computer Group, Madison, WI), DNAsis<sup>TM</sup> (available from Hitachi Software, San Bruno, CA) and MacVector<sup>TM</sup> (available from the Eastman Kodak Company, New Haven, CT). A preferred method to determine percent identity among amino acid sequences and also among nucleic acid sequences includes using the Compare function by maximum matching within the program DNAsis Version 10 2.1 using default parameters.

One embodiment of the present invention includes *Der* HMW-map proteins. In one embodiment, *Der* HMW-map proteins of the present invention include proteins that, when submitted to reducing 12% Tris glycine SDS-PAGE, migrate as bands at a molecular weight of from about 98 kD to about 109 kD, as shown in Fig. 1. The bands 15 in Fig. 1 are obtained when proteins are collected from *Dermataphagoides farinae* mites using the method described in detail in Example 1. Preferably, *Der* HMW-map proteins of the present invention includes proteins having a molecular weight ranging from about 90 kD to about 120 kD, and more preferably from about 98 kD to about 109 kD. Preferred *Der* HMW-map proteins of the present invention include mapA and mapB, the 20 identification of which is described in the Examples section.

In another embodiment, *Der* HMW-map proteins of the present invention include proteins that, when submitted to reducing 14% Tris glycine SDS-PAGE, migrate as a band at a molecular weight of about 60 kD, as shown in Fig. 2. The band in Fig. 2 is obtained when proteins are collected from *Dermataphagoides farinae* mites using the 25 method described in detail in Example 9. Preferably, *Der* HMW-map proteins of the present invention includes proteins having a molecular weight of about 60 kD. Preferred

30 *Der* HMW-map proteins of the present invention include a protein encoded by a nucleic acid molecule which is at least about 50 nucleotides, or about 150

nucleotides, and which hybridizes under conditions which preferably allow about 40% or less base pair mismatch, more preferably under conditions which allow about 35% or less base pair mismatch, more preferably under conditions which allow about 30% or less base pair mismatch, more preferably under conditions which allow about 25% or less base pair mismatch, more preferably under conditions which allow about 20% or less base pair mismatch, more preferably under conditions which allow about 15% or less base pair mismatch, more preferably under conditions which allow about 10% or less base pair mismatch and even more preferably under conditions which allow about 5% or less base pair mismatch with a nucleic acid molecule selected from the group consisting of SEQ ID NO:16, SEQ ID NO:19, SEQ ID NO:22, SEQ ID NO:36, SEQ ID NO:39, SEQ ID NO:42, SEQ ID NO:45 and a nucleic acid sequence encoding a protein comprising the amino acid sequence SEQ ID NO:33 the complement thereof.

Another embodiment of the present invention includes a *Der* HMW-map protein encoded by a nucleic acid molecule selected from the group consisting of: a nucleic acid molecule comprising at least about 150 nucleotides, wherein said nucleic acid molecule comprising at least about 150 nucleotides hybridizes, in a solution comprising 1X SSC and 0% formamide, at a temperature of about 50°C, to a nucleic acid sequence selected from the group consisting of SEQ ID NO:16, SEQ ID NO:19, SEQ ID NO:22, SEQ ID NO:36, SEQ ID NO:39, SEQ ID NO:42, SEQ ID NO:45, and a complement of a nucleic acid sequence encoding a protein comprising the amino acid sequence SEQ ID NO:33; and a nucleic acid molecule comprising a fragment of any of said nucleic acid molecules comprising at least about 15 nucleotides.

Yet another preferred *Der* HMW-map protein of the present invention includes a protein encoded by a nucleic acid molecule which is preferably at least about 60% identical, more preferably at least about 65% identical, more preferably at least about 70% identical, more preferably at least about 75% identical, more preferably at least about 80% identical, more preferably at least about 85% identical, more preferably at least about 90% identical and even more preferably at least about 95% identical to a nucleic acid molecule having the nucleic acid sequence SEQ ID NO:14, SEQ ID NO:17, SEQ ID NO:20, SEQ ID NO:34, SEQ ID NO:37, SEQ ID NO:40, SEQ ID NO:43, and/or a complement of a nucleic acid sequence encoding a protein comprising the

amino acid sequence SEQ ID NO:33; also preferred are fragments of such proteins. Percent identity as used herein is determined using the Compare function by maximum matching within the program DNAsis Version 2.1 using default parameters.

Additional preferred *Der* HMW-map proteins of the present invention include  
5 proteins having the amino acid sequence SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:18, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID  
10 NO:35, SEQ ID NO:38, SEQ ID NO:41, SEQ ID NO:44, and proteins comprising homologs of a protein having the amino acid sequence SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:18, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:24, SEQ  
15 ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:38, SEQ ID NO:41, SEQ ID NO:44 in which such a homolog comprises at least one epitope that elicits an immune response against a protein having an amino acid sequence SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID  
20 NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:18, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:38, SEQ ID NO:41, SEQ ID NO:44. Likewise, also preferred are proteins encoded by nucleic acid molecules encoded by nucleic acid molecules having nucleic acid  
25 sequence SEQ ID NO:14, SEQ ID NO:17, SEQ ID NO:20, SEQ ID NO:34, SEQ ID NO:37, SEQ ID NO:40, SEQ ID NO:43 and/or a nucleic acid sequence encoding a protein comprising the amino acid sequence SEQ ID NO:33, or by homologs thereof.

30 nDerp98<sub>1-100</sub>, nDerp98<sub>1-100</sub>, nDerp98<sub>1-100</sub>, nDerf60<sub>1-100</sub>, or allelic variants of any of these nucleic acid molecules. Another preferred isolated protein is encoded by a nucleic acid

molecule having nucleic acid sequence SEQ ID NO:14, SEQ ID NO:17, SEQ ID NO:20, SEQ ID NO:34, SEQ ID NO:37, SEQ ID NO:40, SEQ ID NO:43; or a protein encoded by an allelic variant of any of these listed nucleic acid molecule.

Translation of SEQ ID NO:14, the coding strand of nDorf98<sub>1752</sub>, yields a protein  
5 of about 555 amino acids, denoted herein as PDorf98<sub>555</sub>, the amino acid sequence of which is presented in SEQ ID NO:15, assuming a first in-frame codon extending from nucleotide 1 to nucleotide 3 of SEQ ID NO:14. The complementary strand of SEQ ID NO:14 is presented herein as SEQ ID NO:16. The amino acid sequence of PDorf98<sub>555</sub> is encoded by the nucleic acid molecule nDorf98<sub>1665</sub>, having a coding strand denoted SEQ  
10 ID NO:17 and a complementary strand denoted SEQ ID NO:19. Analysis of SEQ ID NO:15 suggests the presence of a signal peptide spanning from about amino acid 1 through about amino acid 19. The proposed mature protein, denoted herein as PDorf98<sub>536</sub>, contains about 536 amino acids, the sequence of which is represented herein as SEQ ID NO:21, and is encoded by a nucleic acid molecule referred to herein as  
15 nDorf98<sub>1608</sub>, represented by SEQ ID NO:20, the coding strand, and SEQ ID NO:22, the complementary strand.

Translation of SEQ ID NO:34, the coding strand of nDerp98<sub>1621</sub>, yields a protein of about 509 amino acids, denoted herein as PDerp98<sub>509</sub>, the amino acid sequence of which is presented in SEQ ID NO:35, assuming a first in-frame codon extending from  
20 nucleotide 14 to nucleotide 16 of SEQ ID NO:34. The complementary strand of SEQ ID NO:34 is presented herein as SEQ ID NO:36. The amino acid sequence of PDerp98<sub>509</sub> is encoded by the nucleic acid molecule nDerp98<sub>1527</sub>, having a coding strand denoted SEQ ID NO:37 and a complementary strand denoted SEQ ID NO:39. Analysis of SEQ ID NO:35 suggests the presence of a signal peptide spanning from about amino acid 1  
25 through about amino acid 19. The proposed mature protein, denoted herein as PDerp98<sub>490</sub>, contains about 490 amino acids, the sequence of which is represented herein as SEQ ID NO:41, and is encoded by a nucleic acid molecule referred to herein as nDerp98<sub>1470</sub>, represented by SEQ ID NO:40, the coding strand, and SEQ ID NO:42, the complementary strand.

30 Translation of SEQ ID NO:43, the coding strand of nDorf60<sub>510</sub>, a nucleic acid molecule encoding a portion of the *D. farinae* 60-kD antigen protein yields a protein of

about 170 amino acids, denoted herein as PDerf60<sub>170</sub>, the amino acid sequence of which is presented as SEQ ID NO:44, assuming a first in-frame codon extending from nucleotide 1 to nucleotide 3 of SEQ ID NO:43. The complementary sequence to SEQ ID NO:43 is presented herein as SEQ ID NO:45.

5 Preferred *Der* HMW-map proteins of the present invention include proteins that are at least about 45%, preferably at least about 50%, more preferably at least about 55%, even more preferably at least about 60%, even more preferably at least about 65%, even more preferably at least about 70%, even more preferably at least about 75%, even more preferably at least about 80%, even more preferably at least about 85%, even more preferably at least about 90%, and even more preferably about 95% identical to  
 10 PDerf98<sub>555</sub>. More preferred is a *Der* HMW-map protein comprising PDerf98<sub>555</sub>, PDerf98<sub>536</sub>, PDerp98<sub>509</sub>, PDerp98<sub>490</sub>, and/or PDerf60<sub>170</sub>; and proteins encoded by allelic variants of nucleic acid molecules encoding proteins PDerf98<sub>555</sub>, PDerf98<sub>536</sub>, PDerp98<sub>509</sub>, PDerp98<sub>490</sub>, and/or PDerf60<sub>170</sub>.

15 Other preferred *Der* HMW-map proteins of the present invention include proteins having amino acid sequences that are at least about 45%, preferably at least about 50%, more preferably at least about 55%, even more preferably at least about 60%, even more preferably at least about 65%, even more preferably at least about 70%, even more preferably at least about 75%, even more preferably at least about 80%, even more preferably at least about 85%, even more preferably at least about 90%, and even more preferably about 95% identical to amino acid sequence SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:18, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:24, SEQ  
 20 ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:38, SEQ ID NO:41, and/or SEQ ID NO:44. More preferred are *Der* HMW-map proteins comprising amino acid sequences SEQ ID NO:1, SEQ ID NO:2,  
 25 SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:18, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:38, SEQ ID NO:41, and/or SEQ ID NO:44.

30 SEQ ID NO:15, SEQ ID NO:18, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:38, SEQ ID NO:41, and/or SEQ ID NO:44.

NO:35, SEQ ID NO:38, SEQ ID NO:41, and/or SEQ ID NO:44; and *Der* HMW-map proteins encoded by allelic variants of nucleic acid molecules encoding *Der* HMW-map proteins having amino acid sequences SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID  
5 NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:18, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:38, SEQ ID NO:41, and/or SEQ ID NO:44.

In one embodiment of the present invention, *Der* HMW-map proteins comprise  
10 amino acid sequence SEQ ID NO:15, SEQ ID NO:35, and/or SEQ ID NO:44 (including, but not limited to, the proteins consisting of amino acid sequence SEQ ID NO:15, SEQ ID NO:35, and/or SEQ ID NO:44, fragments thereof, fusion proteins and multivalent proteins), and proteins encoded by allelic variants of nucleic acid molecules encoding proteins having amino acid sequence SEQ ID NO:15, SEQ ID NO:35, and/or SEQ ID  
15 NO:44.

In one embodiment, a preferred *Der* HMW-map protein comprises an amino acid sequence of at least about 35 amino acids in length, preferably at least about 50 amino acids in length, more preferably at least about 100 amino acids in length, more preferably at least about 200 amino acids in length, even more preferably at least about  
20 250 amino acids in length. Within this embodiment, a preferred *Der* HMW-map protein of the present invention has an amino acid sequence comprising at least a portion of SEQ ID NO:15. In another embodiment, a preferred *Der* HMW-map protein comprises a full-length protein, i.e., a protein encoded by a full-length coding region.

Additional preferred *Der* HMW-map proteins of the present invention include  
25 proteins encoded by nucleic acid molecules comprising at least a portion of nDerf98<sub>1752</sub>, nDerf98<sub>1665</sub>, nDerf98<sub>1608</sub>, nDerp98<sub>1621</sub>, nDerp98<sub>1527</sub>, nDerp98<sub>1470</sub>, and nDerf60<sub>510</sub>, as well as *Der* HMW-map proteins encoded by allelic variants of such nucleic acid molecules.

Also preferred are *Der* HMW-map proteins encoded by nucleic acid molecules having nucleic acid sequences comprising at least a portion of SEQ ID NO:14, SEQ ID  
30 NO:17, SEQ ID NO:20, SEQ ID NO:34, SEQ ID NO:37, SEQ ID NO:40 SEQ ID



NO:43 and/or a nucleic acid sequence encoding a protein comprising the amino acid sequence SEQ ID NO:33, as well as allelic variants of these nucleic acid molecules.

In another embodiment, a preferred *Der* HMW-map protein of the present invention is encoded by a nucleic acid molecule comprising at least about 12  
5 nucleotides, preferably at least about 16 nucleotides, more preferably at least about 18 nucleotides, more preferably at least about 20 nucleotides, more preferably at least about 25 nucleotides, more preferably at least about 50 nucleotides, more preferably at least about 100 nucleotides, more preferably at least about 350 nucleotides, more preferably at least about 450 nucleotides, more preferably at least about 500 nucleotides, and even  
10 more preferably at least about 800 nucleotides. Within this embodiment is a *Der* HMW-map protein encoded by at least a portion nDerf98<sub>1752</sub>, nDerp98<sub>1621</sub>, and/or nDerf60<sub>510</sub> or by an allelic variant of these nucleic acid molecules. In yet another embodiment, a preferred *Der* HMW-map protein of the present invention is encoded by a nucleic acid molecule comprising an apparently full-length *Der* HMW-map coding region, i.e., a  
15 nucleic acid molecule encoding an apparently full-length *Der* HMW-map protein.

One embodiment of a *Der* HMW-map protein of the present invention is a fusion protein that includes a *Der* HMW-map protein-containing domain attached to one or more fusion segments. Suitable fusion segments for use with the present invention include, but are not limited to, segments that can: enhance a protein's stability; act as an  
20 immunopotentiator to enhance an immune response against a *Der* HMW-map protein, reduce an IgE response against a *Der* HMW-map protein; and/or assist purification of a *Der* HMW-map protein (e.g., by affinity chromatography). A suitable fusion segment can be a domain of any size that has the desired function (e.g., imparts increased stability, imparts increased immunogenicity to a protein, reduces an IgE response, and/or  
25 simplifies purification of a protein). Fusion segments can be joined to amino and/or carboxyl termini of the *Der* HMW-map protein-containing domain of the protein and can be susceptible to cleavage in order to enable straight-forward recovery of a *Der* HMW-map protein-containing domain. Preferred fusion segments include a metal-binding domain, a fusion segment attached to either the carboxyl and/or amino terminal end of a *Der* HMW-map protein-containing domain. Preferred fusion segments include a metal-binding domain, a fusion segment attached to either the carboxyl and/or amino terminal end of a *Der* HMW-map protein-containing domain. Preferred fusion segments include a metal-binding domain, a fusion segment attached to either the carboxyl and/or amino terminal end of a *Der* HMW-map protein-containing domain.

binding domain (e.g., a poly-histidine segment); an immunoglobulin binding domain (e.g., Protein A; Protein G; T cell; B cell; Fc receptor or complement protein antibody-binding domains); a sugar binding domain (e.g., a maltose binding domain); a "tag" domain (e.g., at least a portion of  $\beta$ -galactosidase, a strep tag peptide, other domains that  
5 can be purified using compounds that bind to the domain, such as monoclonal antibodies); and/or a linker and enzyme domain (e.g., alkaline phosphatase domain connected to a *Der* HMW-map protein by a linker). More preferred fusion segments include metal binding domains, such as a poly-histidine segment; a maltose binding domain; a strep tag peptide, such as that available from Biometra in Tampa, FL; and a  
10 phage T7 S10 peptide.

In another embodiment, a *Der* HMW-map protein of the present invention also includes at least one additional protein segment that is capable of desensitizing an animal from one or more allergens. Such a multivalent desensitizing protein can be produced by culturing a cell transformed with a nucleic acid molecule comprising two or  
15 more nucleic acid domains joined together in such a manner that the resulting nucleic acid molecule is expressed as a multivalent desensitizing compound containing at least two desensitizing compounds capable of desensitizing an animal from allergens.

Examples of multivalent desensitizing compounds include, but are not limited to, a *Der* HMW-map protein of the present invention attached to one or more compounds  
20 that desensitize against allergies caused by one or more allergens, such as a plant allergen, an animal allergen, a parasite allergen or an ectoparasite allergen, including, but not limited to: plant allergens from grass, Meadow Fescue, Curly Dock, plantain, Mexican Firebush, Lamb's Quarters, pigweed, ragweed, sage, elm, cocklebur, Box Elder, walnut, cottonwood, ash, birch, cedar, oak, mulberry, cockroach,  
25 *Dermatophagoides*, *Alternaria*, *Aspergillus*, *Cladosporium*, *Fusarium*, *Helminthosporium*, *Mucor*, *Penicillium*, *Pullularia*, *Rhizopus* and/or *Tricophyton*; parasite allergens from helminths; or ectoparasite allergens from arachnids, insects and leeches, including fleas, ticks, flies, mosquitos, sand flies, black flies, horse flies, horn flies, deer flies, tsetse flies, stable flies, myiasis-causing flies and biting gnats, ants,  
30 spiders, lice; mites and true bugs.

The present invention also includes mimetopes of a *Der* HMW-map protein of the present invention. As used herein, a mimetope of a *Der* HMW-map protein of the present invention refers to any compound that is able to mimic the activity of such a *Der* HMW-map protein (e.g., ability to bind to induce an immune response against *Der* HMW-map protein), often because the mimetope has a structure that mimics the *Der* HMW-map protein. It is to be noted, however, that the mimetope need not have a structure similar to a *Der* HMW-map protein as long as the mimetope functionally mimics the protein. Mimetopes can be, but are not limited to: peptides that have been modified to decrease their susceptibility to degradation; anti-idiotypic and/or catalytic antibodies, or fragments thereof; non-proteinaceous immunogenic portions of an isolated protein (e.g., carbohydrate structures); synthetic or natural organic or inorganic molecules, including nucleic acids; and/or any other peptidomimetic compounds. Mimetopes of the present invention can be designed using computer-generated structures of *Der* HMW-map protein of the present invention. Mimetopes can also be obtained by generating random samples of molecules, such as oligonucleotides, peptides or other organic molecules, and screening such samples by affinity chromatography techniques using the corresponding binding partner, (e.g., an anti-*Der* HMW-map protein antibody). A mimetope can also be obtained by, for example, rational drug design. In a rational drug design procedure, the three-dimensional structure of a compound of the present invention can be analyzed by, for example, nuclear magnetic resonance (NMR) or x-ray crystallography. The three-dimensional structure can then be used to predict structures of potential mimetopes by, for example, computer modeling. The predicted mimetope structures can then be produced by, for example, chemical synthesis, recombinant DNA technology, or by isolating a mimetope from a natural source. Specific examples of *Der* HMW-map protein mimetopes include anti-idiotypic antibodies, oligonucleotides produced using Selex™ technology, peptides identified by random screening of peptide libraries and proteins identified by phage display technology. A preferred mimetope is a map protein that induces an immune response.

The present invention also includes muteins of a *Der* HMW-map protein of the present invention. As used herein, a mutein refers to a particular homolog of a *Der* HMW-map protein in which desired amino acid residues have been substituted or removed. Preferred muteins of the present invention include *Der* HMW-map protein  
5 homologs in which amino acid residues have been changed to reduce an anaphylactic reaction by an animal when the mutein is administered to the animal in therapeutic doses. More preferred muteins of the present invention include *Der* HMW-map protein homologs in which one or more cysteine residues of a *Der* HMW-map protein have been replaced or removed. Methods to produce muteins are known to those of skill in the art  
10 and are disclosed herein. Preferably, a mutein is produced using recombinant techniques.

Another embodiment of the present invention is an isolated nucleic acid molecule comprising a *Der* HMW-map nucleic acid molecule. The identifying characteristics of such nucleic acid molecules are heretofore described. A nucleic acid molecule of the present invention can include an isolated natural *Der* HMW-map gene  
15 or a homolog thereof, the latter of which is described in more detail below. A nucleic acid molecule of the present invention can include one or more regulatory regions, full-length or partial coding regions, or combinations thereof. The minimal size of a nucleic acid molecule of the present invention is a size sufficient to allow the formation of a stable hybrid (i.e., hybridization under stringent hybridization conditions) with the  
20 complementary sequence of another nucleic acid molecule.

In accordance with the present invention, an isolated nucleic acid molecule is a nucleic acid molecule that has been removed from its natural milieu (i.e., that has been subjected to human manipulation) and can include DNA, RNA, or derivatives of either DNA or RNA. As such, "isolated" does not reflect the extent to which the nucleic acid  
25 molecule has been purified. An isolated *Der* HMW-map nucleic acid molecule of the present invention, or a homolog thereof, can be isolated from its natural source or produced using recombinant DNA technology (e.g., polymerase chain reaction (PCR) amplification or cloning) or chemical synthesis. Isolated *Der* HMW-map nucleic acid molecules, and homologs thereof, can include, for example, natural allelic variants and  
30 nucleic acid molecules modified by nucleotide insertions, deletions, substitutions, and/or inversions in a manner such that the modifications do not substantially interfere with the

nucleic acid molecule's ability to encode a *Der* HMW-map protein of the present invention.

A *Der* HMW-map nucleic acid molecule homolog can be produced using a number of methods known to those skilled in the art, see, for example, Sambrook et al., 5 1989, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Labs Press; Sambrook et al., *ibid*. For example, nucleic acid molecules can be modified using a variety of techniques including, but not limited to, classic mutagenesis and recombinant DNA techniques such as site-directed mutagenesis, chemical treatment, restriction 10 enzyme cleavage, ligation of nucleic acid fragments, PCR amplification, synthesis of oligonucleotide mixtures and ligation of mixture groups to "build" a mixture of nucleic acid molecules, and combinations thereof. Nucleic acid molecule homologs can be selected by hybridization with a *Der* HMW-map nucleic acid molecule or by screening the function of a protein encoded by the nucleic acid molecule (e.g., ability to elicit an immune response against at least one epitope of a *Der* HMW-map protein or to effect 15 *Der* HMW-map activity).

Allelic variants typically encode proteins having similar activity to that of the protein encoded by the gene to which they are being compared. Allelic variants can also comprise alterations in the 5' or 3' untranslated regions of the gene (e.g., in regulatory 20 control regions). Allelic variants are well known to those skilled in the art and would be expected to be found within a given dust mite since the genome is diploid and/or among a group of two or more dust mites. The present invention also includes variants due to laboratory manipulation, such as, but not limited to, variants produced during polymerase chain reaction amplification.

An isolated nucleic acid molecule of the present invention can include a nucleic 25 acid sequence that encodes at least one *Der* HMW-map protein of the present invention, examples of such proteins being disclosed herein. Although the phrase "nucleic acid molecule" primarily refers to the physical nucleic acid molecule and the phrase "nucleic

30 nucleic acid molecule, or a nucleic acid sequence, being capable of encoding a *Der* HMW-map protein.

A preferred nucleic acid molecule of the present invention, when administered to an animal, is capable of desensitizing that animal from allergic reactions caused by a *Der* HMW-map allergen. As will be disclosed in more detail below, such a nucleic acid molecule can be, or encode, an antisense RNA, a molecule capable of triple helix formation, a ribozyme, or other nucleic acid-based drug compound. In additional embodiments, a nucleic acid molecule of the present invention can encode a desensitizing protein (e.g., a *Der* HMW-map protein of the present invention), the nucleic acid molecule being delivered to the animal, for example, by direct injection (i.e., as a DNA reagent) or in a vehicle such as a recombinant virus reagent or a recombinant cell reagent.

One embodiment of the present invention is an isolated nucleic acid molecule that hybridizes under stringent hybridization conditions with a *Der* HMW-map gene. Stringent hybridization conditions refer to standard hybridization conditions described herein. A preferred nucleic acid molecule of the present invention includes an isolated nucleic acid molecule that hybridizes under stringent hybridization conditions with a gene encoding a protein comprising an amino acid sequence including SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:18, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:38, SEQ ID NO:41, and/or SEQ ID NO:44. A more preferred nucleic acid molecule of the present invention includes an isolated nucleic acid molecule that hybridizes under stringent hybridization conditions with the complement of a nucleic acid sequence that encodes a protein comprising an amino acid sequence including SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:18, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:38, SEQ ID NO:41, and/or SEQ ID NO:44.

A more preferred nucleic acid molecule of the present invention includes an isolated nucleic acid molecule selected from the group consisting of: a nucleic acid molecule comprising at least about 150 nucleotides, wherein said nucleic acid molecule comprising at least about 150 nucleotides hybridizes, in a solution comprising 1X SSC and 0% formamide, at a temperature of about 50°C, to a nucleic acid sequence selected from the group consisting of SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:43, SEQ ID NO:45 and/or a nucleic acid sequence encoding a protein comprising the amino acid sequence SEQ ID NO:33 and a complement thereof.

The present invention also includes fragments of any nucleic acid molecule disclosed herein. According to the present invention, a fragment can include any nucleic acid molecule or nucleic acid sequence, the size of which can range between a length that is smaller than a sequence identified by a SEQ ID NO of the present invention and the minimum size of an oligonucleotide as defined herein. For example, the size of a fragment of the present invention can be any size that is less than about 1752 nucleotides and greater than 11 nucleotides in length.

In one embodiment of the present invention, a preferred *Der* HMW-map nucleic acid molecule includes an isolated nucleic acid molecule which is at least about 50 nucleotides, or at least about 150 nucleotides, and which hybridizes under conditions which preferably allow about 40% or less base pair mismatch, more preferably under conditions which allow about 35% or less base pair mismatch, more preferably under conditions which allow about 30% or less base pair mismatch, more preferably under conditions which allow about 25% or less base pair mismatch, more preferably under conditions which allow about 20% or less base pair mismatch, more preferably under conditions which allow about 15% or less base pair mismatch, more preferably under conditions which allow about 10% or less base pair mismatch and even more preferably

SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:43, SEQ ID

NO:45, and a nucleic acid sequence encoding a protein comprising the amino acid sequence SEQ ID NO:33 and a complement thereof.

Another embodiment of the present invention includes a nucleic acid molecule comprising at least about 150 base-pairs, wherein the nucleic acid molecule hybridizes, in a solution comprising 1X SSC and 0% formamide, at a temperature of about 50°C, to a nucleic acid sequence selected from the group consisting of SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:43, SEQ ID NO:45, and/or a nucleic acid sequence encoding a protein comprising the amino acid sequence SEQ ID NO:33 and a complement thereof. Additional preferred nucleic acid molecules of the present invention include fragments of an isolated nucleic acid molecule comprising at least about 150 base-pairs, wherein said nucleic acid molecule hybridizes, in a solution comprising 1X SSC and 0% formamide, at a temperature of about 50°C, to a nucleic acid sequence selected from the group consisting of SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:43, SEQ ID NO:45 and a nucleic acid sequence encoding a protein comprising the amino acid sequence SEQ ID NO:33 and complement thereof.

Additional preferred *Der* HMW-map nucleic acid molecules of the present invention include an isolated nucleic acid molecule which is at least about 50 nucleotides, or at least about 150 nucleotides, comprising a nucleic acid sequence that is preferably at least about 60% identical, more preferably at least about 65% identical, more preferably at least about 70% identical, more preferably at least about 75% identical, more preferably at least about 80% identical, more preferably at least about 85% identical, more preferably at least about 90% identical and even more preferably at least about 95% identical to a nucleic acid sequence selected from the group consisting of SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:43, SEQ ID NO:45, and a nucleic acid sequence encoding a protein comprising the amino acid sequence SEQ ID NO:33 and a



complement thereof. Also preferred are fragments of any of such nucleic acid molecules. Percent identity may be determined using the Compare function by maximum matching within the program DNAsis Version 2.1 using default parameters.

One embodiment of the present invention is a nucleic acid molecule comprising  
 5 all or part of nucleic acid molecules nDerf98<sub>1752</sub>, nDerf98<sub>1665</sub> and nDerf98<sub>1608</sub>,  
 nDerp98<sub>1621</sub>, nDerp98<sub>1527</sub>, nDerp98<sub>1470</sub>, and/or nDerf60<sub>510</sub>, or allelic variants of these  
 nucleic acid molecules. Another preferred nucleic acid molecule of the present  
 invention includes at least a portion of nucleic acid sequence SEQ ID NO:14, SEQ ID  
 NO:16, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:22, SEQ ID  
 10 NO:34, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:40, SEQ ID  
 NO:42, SEQ ID NO:43, SEQ ID NO:45 and/or a nucleic acid sequence encoding a  
 protein comprising the amino acid sequence SEQ ID NO:33, as well as allelic variants of  
 nucleic acid molecules having these nucleic acid sequences and homologs of nucleic  
 acid molecules having these nucleic acid sequences; preferably such a homolog encodes  
 15 or is complementary to a nucleic acid molecule that encodes at least one epitope that  
 elicits and an immune response against a protein having an amino acid sequence SEQ ID  
 NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ  
 ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID  
 NO:12, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:18, SEQ ID NO:21, SEQ ID  
 20 NO:23, SEQ ID NO:24, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID  
 NO:32, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:38, SEQ ID NO:41, SEQ ID  
 NO:41, and/or SEQ ID NO:44. Such nucleic acid molecules can include nucleotides in  
 addition to those included in the SEQ ID NOs, such as, but not limited to, a full-length  
 gene, a full-length coding region, a nucleic acid molecule encoding a fusion protein, or a  
 25 nucleic acid molecule encoding a multivalent protective compound.

In one embodiment, a *Der* HMW-map nucleic acid molecule of the present  
 invention encodes a protein that is at least about 45%, preferably at least about 50%

preferably at least about 75%, even more preferably at least about 80%, even more  
 preferably at least about 85%, even more preferably at least about 90%, and even more

preferably about 95% identical to PDerf98<sub>555</sub>, PDerp98<sub>509</sub>, and/or PDerf60<sub>170</sub>. Even more preferred is a nucleic acid molecule encoding PDerf98<sub>555</sub>, PDerf98<sub>536</sub>, PDerp98<sub>509</sub>, PDerp98<sub>490</sub>, and/or PDerf60<sub>170</sub>, and/or an allelic variant of such nucleic acid molecules.

In another embodiment, a *Der* HMW-map nucleic acid molecule of the present invention encodes a protein having an amino acid sequence that is at least about 45%, preferably at least about 50%, more preferably at least about 55%, even more preferably at least about 60%, even more preferably at least about 65%, even more preferably at least about 70%, even more preferably at least about 75%, even more preferably at least about 80%, even more preferably at least about 85%, even more preferably at least about 90%, and even more preferably about 95% identical to SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:18, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:38, SEQ ID NO:41, SEQ ID NO:41, and/or SEQ ID NO:44. The present invention also includes a *Der* HMW-map nucleic acid molecule encoding a protein having at least a portion of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:18, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:38, SEQ ID NO:41, SEQ ID NO:41, and/or SEQ ID NO:44, as well as allelic variants of a *Der* HMW-map nucleic acid molecule encoding a protein having these sequences, including nucleic acid molecules that have been modified to accommodate codon usage properties of the cells in which such nucleic acid molecules are to be expressed.

In another embodiment, a preferred *Der* HMW-map nucleic acid molecule encodes a *Der* HMW-map protein comprising at least about at least about 35 amino acids in length, preferably at least about 50 amino acids in length, more preferably at least about 100 amino acids in length, more preferably at least about 200 amino acids in length, even more preferably at least about 250 amino acids in length.

Knowing the nucleic acid sequences of certain *Der* HMW-map nucleic acid molecules of the present invention allows one skilled in the art to, for example, (a) make copies of those nucleic acid molecules, (b) obtain nucleic acid molecules including at least a portion of such nucleic acid molecules (e.g., nucleic acid molecules including full-length genes, full-length coding regions, regulatory control sequences, truncated coding regions), and (c) obtain other *Der* HMW-map nucleic acid molecules. Such nucleic acid molecules can be obtained in a variety of ways including screening appropriate expression libraries with antibodies of the present invention; traditional cloning techniques using oligonucleotide probes of the present invention to screen appropriate libraries; and PCR amplification of appropriate libraries or DNA using oligonucleotide primers of the present invention. A preferred library to screen or from which to amplify nucleic acid molecules includes a *Dermatophagoides farinae* and/or *Dermatophagoides pteronyssius* library, such as the libraries disclosed herein in the Examples. Techniques to clone and amplify genes are disclosed, for example, in Sambrook et al., *ibid*.

The present invention also includes nucleic acid molecules that are oligonucleotides capable of hybridizing, under stringent hybridization conditions, with complementary regions of other, preferably longer, nucleic acid molecules of the present invention such as those comprising *Der* HMW-map nucleic acid molecules or other *Der* HMW-map nucleic acid molecules. Oligonucleotides of the present invention can be RNA, DNA, or derivatives of either. The minimum size of such oligonucleotides is the size required for formation of a stable hybrid between an oligonucleotide and a complementary sequence on a nucleic acid molecule of the present invention. A preferred oligonucleotide of the present invention has a maximum size of preferably about 200 nucleotides, more preferably about 150 nucleotides and even more preferably about 100 nucleotides. The present invention includes oligonucleotides that can be used as, for example, probes to identify nucleic acid molecules

any vector capable of delivering the nucleic acid molecule into a host cell. Such a vector contains heterologous nucleic acid sequences, that is nucleic acid sequences that are not

naturally found adjacent to nucleic acid molecules of the present invention and that preferably are derived from a species other than the species from which the nucleic acid molecule(s) are derived. The vector can be either RNA or DNA, either prokaryotic or eukaryotic, and typically is a virus or a plasmid. Recombinant vectors can be used in the  
5 cloning, sequencing, and/or otherwise manipulation of *Der* HMW-map nucleic acid molecules of the present invention.

One type of recombinant vector, referred to herein as a recombinant molecule, comprises a nucleic acid molecule of the present invention operatively linked to an expression vector. The phrase operatively linked refers to insertion of a nucleic acid  
10 molecule into an expression vector in a manner such that the molecule is able to be expressed when transformed into a host cell. As used herein, an expression vector is a DNA or RNA vector that is capable of transforming a host cell and of effecting expression of a specified nucleic acid molecule. Preferably, the expression vector is also capable of replicating within the host cell. Expression vectors can be either prokaryotic  
15 or eukaryotic, and are typically viruses or plasmids. Expression vectors of the present invention include any vectors that function (i.e., direct gene expression) in recombinant cells of the present invention, including in bacterial, fungal, endoparasite, insect, other animal, and plant cells. Preferred expression vectors of the present invention can direct gene expression in bacterial, yeast, insect and mammalian cells and more preferably in  
20 the cell types disclosed herein.

In particular, expression vectors of the present invention contain regulatory sequences such as transcription control sequences, translation control sequences, origins of replication, and other regulatory sequences that are compatible with the recombinant cell and that control the expression of nucleic acid molecules of the present invention.  
25 In particular, recombinant molecules of the present invention include transcription control sequences. Transcription control sequences are sequences which control the initiation, elongation, and termination of transcription. Particularly important transcription control sequences are those which control transcription initiation, such as promoter, enhancer, operator and repressor sequences. Suitable transcription control  
30 sequences include any transcription control sequence that can function in at least one of the recombinant cells of the present invention. A variety of such transcription control

sequences are known to those skilled in the art. Preferred transcription control sequences include those which function in bacterial, yeast, insect and mammalian cells, such as, but not limited to, *tac*, *lac*, *trp*, *trc*, oxy-pro, omp/lpp, *rrnB*, bacteriophage lambda (such as lambda  $p_L$  and lambda  $p_R$  and fusions that include such promoters),

5 bacteriophage T7, T7*lac*, bacteriophage T3, bacteriophage SP6, bacteriophage SP01, metallothionein, alpha-mating factor, *Pichia* alcohol oxidase, alphavirus subgenomic promoters (such as Sindbis virus subgenomic promoters), antibiotic resistance gene, baculovirus, *Heliothis zea* insect virus, vaccinia virus, herpesvirus, raccoon poxvirus, other poxvirus, adenovirus, cytomegalovirus (such as intermediate early promoters),

10 simian virus 40, retrovirus, actin, retroviral long terminal repeat, Rous sarcoma virus, heat shock, phosphate and nitrate transcription control sequences as well as other sequences capable of controlling gene expression in prokaryotic or eukaryotic cells. Additional suitable transcription control sequences include tissue-specific promoters and enhancers as well as lymphokine-inducible promoters (e.g., promoters inducible by

15 interferons or interleukins). Transcription control sequences of the present invention can also include naturally occurring transcription control sequences naturally associated with canines or felines.

Suitable and preferred nucleic acid molecules to include in recombinant vectors of the present invention are as disclosed herein. Preferred nucleic acid molecules to

20 include in recombinant vectors, and particularly in recombinant molecules, include nDerf98<sub>1752</sub>, nDerf98<sub>1665</sub>, nDerf98<sub>1608</sub>, nDerp98<sub>1621</sub>, nDerp98<sub>1527</sub>, nDerp98<sub>1470</sub>, and nDerf60<sub>510</sub>.

Recombinant molecules of the present invention may also (a) contain secretory signals (i.e., signal segment nucleic acid sequences) to enable an expressed *Der* HMW-

25 map protein of the present invention to be secreted from the cell that produces the protein and/or (b) contain fusion sequences which lead to the expression of nucleic acid molecules of the present invention as fusion proteins. Examples of suitable signal

30 plasminogen activator ( $\alpha$ -PA), interferon, interleukin, growth hormone, histocompatibility and viral envelope glycoprotein signal segments, as well as natural

signal segments. Suitable fusion segments encoded by fusion segment nucleic acids are disclosed herein. In addition, a nucleic acid molecule of the present invention can be joined to a fusion segment that directs the encoded protein to the proteasome, such as a ubiquitin fusion segment. Recombinant molecules may also include intervening and/or  
5 untranslated sequences surrounding and/or within the nucleic acid sequences of nucleic acid molecules of the present invention.

Another embodiment of the present invention includes a recombinant cell comprising a host cell transformed with one or more recombinant molecules of the present invention. Transformation of a nucleic acid molecule into a cell can be  
10 accomplished by any method by which a nucleic acid molecule can be inserted into the cell. Transformation techniques include, but are not limited to, transfection, electroporation, microinjection, lipofection, adsorption, and protoplast fusion. A recombinant cell may remain unicellular or may grow into a tissue, organ or a multicellular organism. Transformed nucleic acid molecules of the present invention  
15 can remain extrachromosomal or can integrate into one or more sites within a chromosome of the transformed (i.e., recombinant) cell in such a manner that their ability to be expressed is retained. Preferred nucleic acid molecules with which to transform a cell include *Der* HMW-map nucleic acid molecules disclosed herein. Particularly preferred nucleic acid molecules with which to transform a cell include  
20 nDerf98<sub>1752</sub>, nDerf98<sub>1665</sub>, nDerf98<sub>1608</sub>, nDerp98<sub>1621</sub>, nDerp98<sub>1527</sub>, nDerp98<sub>1470</sub>, and nDerf60<sub>510</sub>.

Suitable host cells to transform include any cell that can be transformed with a nucleic acid molecule of the present invention. Host cells can be either untransformed cells or cells that are already transformed with at least one nucleic acid molecule (e.g.,  
25 nucleic acid molecules encoding one or more proteins of the present invention and/or other proteins useful in the production of multivalent vaccines). Host cells of the present invention either can be endogenously (i.e., naturally) capable of producing *Der* HMW-map proteins of the present invention or can be capable of producing such proteins after being transformed with at least one nucleic acid molecule of the present invention. Host  
30 cells of the present invention can be any cell capable of producing at least one protein of the present invention, and include bacterial, fungal (including yeast), other insect, other

animal and plant cells. Preferred host cells include bacterial, mycobacterial, yeast, parasite, insect and mammalian cells. More preferred host cells include *Salmonella*, *Escherichia*, *Bacillus*, *Listeria*, *Saccharomyces*, *Spodoptera*, *Mycobacteria*, *Trichoplusia*, BHK (baby hamster kidney) cells, MDCK cells (normal dog kidney cell line for canine herpesvirus cultivation), CRFK cells (normal cat kidney cell line for feline herpesvirus cultivation), CV-1 cells (African monkey kidney cell line used, for example, to culture raccoon poxvirus), COS (e.g., COS-7) cells, and Vero cells. Particularly preferred host cells are *Escherichia coli*, including *E. coli* K-12 derivatives; *Salmonella typhi*; *Salmonella typhimurium*, including attenuated strains such as UK-1 x3987 and SR-11 x4072; *Spodoptera frugiperda*; *Trichoplusia ni*; BHK cells; MDCK cells; CRFK cells; CV-1 cells; COS cells; Vero cells; and non-tumorigenic mouse myoblast G8 cells (e.g., ATCC CRL 1246). Additional appropriate mammalian cell hosts include other kidney cell lines, other fibroblast cell lines (e.g., human, murine or chicken embryo fibroblast cell lines), myeloma cell lines, Chinese hamster ovary cells, mouse NIH/3T3 cells, LMTK<sup>31</sup> cells and/or HeLa cells.

A recombinant cell is preferably produced by transforming a host cell with one or more recombinant molecules, each comprising one or more nucleic acid molecules of the present invention operatively linked to an expression vector containing one or more transcription control sequences. The phrase operatively linked refers to insertion of a nucleic acid molecule into an expression vector in a manner such that the molecule is able to be expressed when transformed into a host cell.

A recombinant molecule of the present invention is a molecule that can include at least one of any nucleic acid molecule heretofore described operatively linked to at least one of any transcription control sequence capable of effectively regulating expression of the nucleic acid molecule(s) in the cell to be transformed, examples of which are disclosed herein.

A recombinant cell of the present invention includes any cell transformed with at least one of the preferred recombinant molecules with which to transform cells are disclosed herein.

Recombinant DNA technologies can be used to improve expression of transformed nucleic acid molecules by manipulating, for example, the number of copies of the nucleic acid molecules within a host cell, the efficiency with which those nucleic acid molecules are transcribed, the efficiency with which the resultant transcripts are translated, and the efficiency of post-translational modifications. Recombinant techniques useful for increasing the expression of nucleic acid molecules of the present invention include, but are not limited to, operatively linking nucleic acid molecules to high-copy number plasmids, integration of the nucleic acid molecules into one or more host cell chromosomes, addition of vector stability sequences to plasmids, substitutions or modifications of transcription control signals (e.g., promoters, operators, enhancers), substitutions or modifications of translational control signals (e.g., ribosome binding sites, Shine-Dalgarno sequences), modification of nucleic acid molecules of the present invention to correspond to the codon usage of the host cell, deletion of sequences that destabilize transcripts, and use of control signals that temporally separate recombinant cell growth from recombinant enzyme production during fermentation. The activity of an expressed recombinant protein of the present invention may be improved by fragmenting, modifying, or derivatizing nucleic acid molecules encoding such a protein.

Isolated *Der* HMW-map proteins of the present invention can be produced in a variety of ways, including production and recovery of natural proteins, production and recovery of recombinant proteins, and chemical synthesis of the proteins. In one embodiment, an isolated protein of the present invention is produced by culturing a cell capable of expressing the protein under conditions effective to produce the protein, and recovering the protein. A preferred cell to culture is a recombinant cell of the present invention. Effective culture conditions include, but are not limited to, effective media, bioreactor, temperature, pH and oxygen conditions that permit protein production. An effective medium refers to any medium in which a cell is cultured to produce a *Der* HMW-map protein of the present invention. Such a medium typically comprises an aqueous medium having assimilable carbon, nitrogen and phosphate sources, and appropriate salts, minerals, metals and other nutrients, such as vitamins. Cells of the present invention can be cultured in conventional fermentation bioreactors, shake flasks, test tubes, microtiter dishes, and petri plates. Culturing can be carried out at a



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temperature, pH and oxygen content appropriate for a recombinant cell. Such culturing conditions are within the expertise of one of ordinary skill in the art.

Depending on the vector and host system used for production, resultant proteins of the present invention may either remain within the recombinant cell; be secreted into the fermentation medium; be secreted into a space between two cellular membranes, such as the periplasmic space in *E. coli*; or be retained on the outer surface of a cell or viral membrane. The phrase "recovering the protein", as well as similar phrases, refers to collecting the whole fermentation medium containing the protein and need not imply additional steps of separation or purification. Proteins of the present invention can be purified using a variety of standard protein purification techniques, such as, but not limited to, affinity chromatography, ion exchange chromatography, filtration, electrophoresis, hydrophobic interaction chromatography, gel filtration chromatography, reverse phase chromatography, concanavalin A chromatography, chromatofocusing and differential solubilization. Proteins of the present invention are preferably retrieved in "substantially pure" form. As used herein, "substantially pure" refers to a purity that allows for the effective use of the protein as a therapeutic composition or diagnostic. A therapeutic composition for animals, for example, should exhibit no substantial toxicity and preferably should be capable of desensitizing a treated animal.

The present invention also includes isolated (i.e., removed from their natural milieu) antibodies that selectively bind to a *Der* HMW-map protein of the present invention or a mimetope thereof (i.e., anti-*Der* HMW-map protein antibodies). As used herein, the term "selectively binds to" a *Der* HMW-map protein refers to the ability of antibodies of the present invention to preferentially bind to specified proteins and mimetopes thereof of the present invention. Binding can be measured using a variety of methods standard in the art including enzyme immunoassays (e.g., ELISA), immunoblot assays, etc.; see, for example, Sambrook et al., *ibid*. An anti-*Der* HMW-map protein antibody preferably selectively binds to a portion of a *Der* HMW-map protein that

fluid (such as, but not limited to, serum), or antibodies that have been purified to varying degrees. Antibodies of the present invention can be polyclonal or monoclonal

Functional equivalents of such antibodies, such as antibody fragments and genetically-engineered antibodies (including single chain antibodies or chimeric antibodies that can bind to more than one epitope) are also included in the present invention.

A preferred method to produce antibodies of the present invention includes

5 (a) administering to an animal an effective amount of a protein, peptide or mimotope thereof of the present invention to produce the antibodies and (b) recovering the antibodies. In another method, antibodies of the present invention are produced recombinantly using techniques as heretofore disclosed to produce *Der* HMW-map proteins of the present invention. Antibodies raised against defined proteins or

10 mimetopes can be advantageous because such antibodies are not substantially contaminated with antibodies against other substances that might otherwise cause interference in a diagnostic assay or side effects if used in a therapeutic composition.

Antibodies of the present invention have a variety of potential uses that are within the scope of the present invention. For example, such antibodies can be used (a)

15 as tools to detect mite allergen, in particular *Der* HMW-map protein; (b) as tools to screen expression libraries; and/or (c) to recover desired proteins of the present invention from a mixture of proteins and other contaminants. Antibodies of the present invention can also be used, for example, to inhibit binding of *Der* HMW-map protein to IgE that binds specifically to *Der* HMW-map protein, to prevent immunocomplex formation,

20 thereby reducing hypersensitivity responses to mite allergens.

A *Der* HMW-map protein of the present invention can be included in a chimeric molecule comprising at least a portion of a *Der* HMW-map protein that induces an immune response in an animal and a second molecule that enables the chimeric molecule to be bound to a substrate in such a manner that the *Der* HMW-map protein

25 portion can bind to IgE in essentially the same manner as a *Der* HMW-map protein that is not bound to a substrate. An example of a suitable second molecule includes a portion of an immunoglobulin molecule or another ligand that has a suitable binding partner that can be immobilized on a substrate, e.g., biotin and avidin, or a metal-binding protein and a metal (e.g., His), or a sugar-binding protein and a sugar (e.g., maltose).

30 A *Der* HMW-map protein of the present invention can be contained in a formulation, herein referred to as a *Der* HMW-map protein formulation. For example, a

*Der* HMW-map protein can be combined with a buffer in which the *Der* HMW-map protein is solubilized, and/or with a carrier. Suitable buffers and carriers are known to those skilled in the art. Examples of suitable buffers include any buffer in which a *Der* HMW-map protein can function to selectively bind to an antibody that specifically binds to *Der* HMW-map protein, such as, but not limited to, phosphate buffered saline, water, saline, phosphate buffer, bicarbonate buffer, HEPES buffer (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid buffered saline), TES buffer (Tris-EDTA buffered saline), Tris buffer and TAE buffer (Tris-acetate-EDTA). Examples of carriers include, but are not limited to, polymeric matrices, toxoids, and serum albumins, such as bovine serum albumin. Carriers can be mixed with *Der* HMW-map protein or conjugated (i.e., attached) to *Der* HMW-map protein in such a manner as to not substantially interfere with the ability of the *Der* HMW-map protein to selectively bind to an antibody that specifically binds to *Der* HMW-map protein.

A *Der* HMW-map protein of the present invention can be produced by a cell comprising the *Der* HMW-map protein. A preferred *Der* HMW-map protein-bearing cell includes a recombinant cell comprising a nucleic acid molecule encoding a *Der* HMW-map protein of the present invention.

In addition, a *Der* HMW-map protein formulation of the present invention can include not only a *Der* HMW-map protein but also one or more additional antigens or antibodies useful in desensitizing an animal against allergy, or preventing or treating mite allergen pathogenesis. As used herein, an antigen refers to any molecule capable of being selectively bound by an antibody. As used herein, an allergen refers to any antigen that is capable of stimulating production of antibodies involved in an allergic response in an animal. As used herein, selective binding of a first molecule to a second molecule refers to the ability of the first molecule to preferentially bind (e.g., having higher affinity higher avidity) to the second molecule when compared to the ability of a first molecule to bind to a third molecule. The first molecule need not necessarily be the

allergens and plant allergens

In accordance with the present invention, virtually any substance can act as an antigen and elicit an antibody response, i.e., can function as an epitope. For example, antibodies can be raised in response to carbohydrate epitopes, including saccharides and/or polysaccharides that are attached to a protein, a so-called glycosylated protein.

5 However, a saccharide and/or polysaccharide may act as an antigen alone, without a protein being present. The terminal sugar of a carbohydrate moiety, as well as internal sugars can serve as an epitope. Polysaccharide may be present as a branched chain, in which case epitopes may comprise sugars that are not contiguous in sequence, but are adjacent spatially. Unusual, insect-specific sugars, not normally seen in mammalian

10 proteins, may be present on glycoprotein derived from insect nucleic acid molecules, and these unusual sugars can comprise an epitope recognized by a mammalian immune system.

One embodiment of the present invention is a reagent comprising a non-proteinaceous epitope that is capable of binding to IgE of an animal that is allergic to

15 mites, of desensitizing an animal against mite allergen, of stimulating a B lymphocyte response, and/or of stimulating a T lymphocyte response. Such an epitope, referred to herein as a *Der* NP epitope, can exist as part of a *Der* HMW-map protein of the present invention or can be isolated therefrom. Such an epitope exists, for example, on a protein contained in the *D. farinae* HMW-map composition produced in accordance with

20 Example 1. A *Der* NP epitope of the present invention can be isolated from its natural source or produced synthetically. Such an epitope can be, but need not be, joined to a carrier or other molecule. A *Der* NP epitope has at least one of the following identifying characteristics: (a) the epitope is resistant to  $\beta$ -elimination of peptides; (b) the epitope is resistant to Proteinase-K digestion; and (c) the epitope is reactive to a test designed to

25 detect glycosylated proteins. A preferred *Der* NP epitope has all such identifying characteristics. A *Der* NP epitope can selectively bind to IgE of dogs or cats that are allergic to mites. While not being bound by theory, it is believed that a *Der* NP epitope comprises a carbohydrate moiety that apparently does not include an N-linked glycan. Identification of the structural characteristics of such an epitope can be determined by

30 one skilled in the art. In one embodiment, there is provided an isolated antibody that selectively binds to a *Der* NP epitope. The present invention also includes a derivative

of a *Der* NP epitope, i.e., a compound that mimics the activity of such an epitope (e.g. is a *Der* NP epitope mimetope) and is capable of binding to antibody raised against a native (i.e. seen in nature) *Der* NP epitope.

A reagent comprising a *Der* NP epitope of the present invention can be used in a  
5 variety of ways in accordance with the present invention. Such a reagent can be a desensitizing compound or a detection reagent to test for mite allergy susceptibility or sensitivity. In one embodiment, a therapeutic composition of the present invention includes a reagent comprising a *Der* NP epitope. In another embodiment, an assay kit of the present invention includes a reagent comprising a *Der* NP epitope. One embodiment  
10 of the present invention is a method to identify an animal susceptible to or having an allergic response to a mite. Such a method includes the steps of contacting a reagent comprising a *Der* NP epitope with antibodies of an animal and determining immunocomplex formation between the reagent and the antibodies, wherein formation of the immunocomplex indicates that the animal is susceptible to or has said allergic  
15 response. Another embodiment of the present invention is a method to desensitize a host animal to an allergic response to a mite. Such a method includes the step of administering to the animal a therapeutic composition that includes a reagent comprising a *Der* NP epitope as a desensitizing compound.

Another embodiment of the present invention is a *Der* HMW-map protein  
20 lacking *Der* NP epitopes. Without being bound by theory, it is believed that such a protein would be a better desensitizing compound since such a protein is expected to have a reduced ability to bind to IgE. Such a protein can be produced by, for example, removing *Der* NP epitopes from a native *Der* HMW-map protein or by producing the protein recombinantly, for example in *E. coli*.

25 One embodiment of the present invention is an *in vivo* test that is capable of detecting whether an animal is hypersensitive to *Der* HMW-map protein. An *in vivo* hypersensitivity test of the present invention is particularly useful for identifying animals

30 administering (e.g. intradermally injecting or superficial scratching) an effective amount of a formulation containing *Der* HMW-map protein, or a mimetope thereof. Methods to

conduct skin tests of the present invention are known to those of skill in the art and are briefly disclosed herein.

Suitable formulations to use in an *in vivo* skin test include *Der* HMW-map protein, homologs of *Der* HMW-map protein and/or mimetopes of *Der* HMW-map  
5 protein

It is understood by one of skill in the art that a suitable amount of *Der* HMW-map protein formulation for use in a skin test of the present invention can vary widely depending on the allergenicity of the formulation used in the test and on the site at which the product is delivered. Suitable amounts of *Der* HMW-map protein formulation for  
10 use in a skin test of the present invention include an amount capable of forming reaction, such as a detectable wheal or induration (hardness) resulting from an allergic reaction to the formulation. Preferred amounts of *Der* HMW-map protein for use in a skin test of the present invention range from about  $1 \times 10^{-8}$  micrograms ( $\mu\text{g}$ ) to about 100  $\mu\text{g}$ , more preferably from about  $1 \times 10^{-7}$   $\mu\text{g}$  to about 10  $\mu\text{g}$ , and even more preferably from about  $1 \times 10^{-6}$   $\mu\text{g}$  to about 1  $\mu\text{g}$  of *Der* HMW-map protein. It is to be appreciated by those of  
15 skill in the art that such amounts will vary depending upon the allergenicity of the protein being administered.

According to the present invention, *Der* HMW-map protein of the present invention can be combined with an immunopotentiator (e.g., carriers or adjuvants of the  
20 present invention as defined in detail below). A novel aspect, however, of the present invention is that *Der* HMW-map protein of the present invention can induce a hypersensitive response in the absence of an immunopotentiator, particularly in canines.

A skin test of the present invention further comprises administering a control solution to an animal. A control solution can include a negative control solution and/or  
25 a positive control solution. A positive control solution of the present invention contains an effective amount of at least one compound known to induce a hypersensitive response when administered to an animal. A preferred compound for use as positive control solution includes, but is not limited to, histamine. A negative control solution of the present invention can comprise a solution that is known not to induce a hypersensitive  
30 response when administered to an animal. As such, a negative control solution can comprise a solution having compounds essentially incapable of inducing a

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hypersensitive response or simply a buffer used to prepare the formulation, such as saline. An example of a preferred negative control solution is phenolated phosphate buffered saline (available from Greer Laboratories, Inc., Lenoir, NC).

Hypersensitivity of an animal to one or more formulations of the present invention can be evaluated by measuring reactions (e.g., wheal size, induration or hardness; using techniques known to those skilled in the art) resulting from administration of one or more experimental sample(s) and control sample(s) into an animal and comparing the reactions to the experimental sample(s) with reactions resulting from administration of one or more control solution. Preferred devices for intradermal injections include individual syringes. Preferred devices for scratching include devices that permit the administration of a number of samples at one time. The hypersensitivity of an animal can be evaluated by determining if the reaction resulting from administration of a formulation of the present invention is larger than the reaction resulting from administration of a negative control, and/or by determining if the reaction resulting from administration of the formulation is at least about the same size as the reaction resulting from administration of a positive control solution. As such, if an experimental sample produces a reaction greater than or equal to the size of a wheal produced by administration of a positive control sample to an animal, then that animal is hypersensitive to the experimental sample. Conversely, if an experimental sample produces a reaction similar to the reaction produced by administration of a negative control sample to an animal, then that animal is not hypersensitive to the experimental sample.

Preferred wheal sizes for evaluation of the hypersensitivity of an animal range from about 16 mm to about 8 mm, more preferably from about 15 mm to about 9 mm, and even more preferably from about 14 mm to about 10 mm in diameter.

Preferably, the ability or inability of an animal to exhibit an immediate hypersensitive response to a formulation of the present invention is determined by

administration of a sample, and even more preferably about 15 minutes after administration of a sample.

Preferably, the ability or inability of an animal to exhibit a delayed hypersensitive response to a formulation of the present invention is determined by measuring induration and/or erythema from about 18 hours to about 30 hours after administration of a sample, more preferably from about 20 hours to about 28 hours after administration of a sample, and even more preferably at about 24 hours after administration of a sample. A delayed hypersensitivity response can also be measured using other techniques such as by determining, using techniques known to those of skill in the art, the extent of cell infiltrate at the site of administration during the time periods defined directly above.

In a preferred embodiment, a skin test of the present invention comprises intradermally injecting into an animal at a given site an effective amount of a formulation that includes *Der* HMW-map protein, and intradermally injecting an effective amount of a control solution into the same animal at a different site. It is within the scope of one of skill in the art to use devices capable of delivering multiple samples simultaneously at a number of sites, preferably enabling concurrent evaluation of numerous formulations. A preferred *Der* HMW-map protein for use with a skin test includes full-length protein. A preferred positive control sample can be a sample comprising histamine. A preferred negative control sample can be a sample comprising diluent.

Animals suitable and preferred to test for hypersensitivity to *Der* HMW-map protein using a skin test of the present invention are disclosed herein. Particularly preferred animals to test with a skin test of the present invention include humans, canines, felines and equines, with human, canines and felines being even more preferred. As used herein, canine refers to any member of the dog family, including domestic dogs, wild dogs and zoo dogs. Examples of dogs include, but are not limited to, domestic dogs, wild dogs, foxes, wolves, jackals and coyotes. As used herein, feline refers to any member of the cat family, including domestic cats, wild cats and zoo cats. Examples of cats include, but are not limited to, domestic cats, lions, tigers, leopards, panthers, cougars, bobcats, lynx, jaguars, cheetahs and servals. As used herein, equine refers to any member of the horse family, including horses, donkeys, mules and zebras.

One embodiment of the present invention is a method to detect antibodies *in vitro* that bind to *Der* HMW-map protein (referred to herein as anti-*Der* HMW-map



antibody) which includes the steps of: (a) contacting an isolated *Der* HMW-map protein with a putative anti-*Der* HMW-map antibody-containing composition under conditions suitable for formation of a *Der* HMW-map protein:antibody complex; and (b) detecting the presence of the antibody by detecting the *Der* HMW-map protein:antibody complex.

5 Presence of such a *Der* HMW-map protein:antibody complex indicates that the animal is producing antibody to a mite allergen. Preferred anti-*Der* HMW-map antibody to detect include antibodies having an IgE or IgG isotype. Preferred anti-*Der* HMW-map antibody to detect include feline antibody, canine antibody, equine antibody and human antibody, with feline, canine and human antibody being particularly preferred.

10 As used herein, the term "contacting" refers to combining or mixing, in this case a putative antibody-containing composition with a *Der* HMW-map protein. Formation of a complex between a *Der* HMW-map protein and an antibody refers to the ability of the *Der* HMW-map protein to selectively bind to the antibody in order to form a stable complex that can be measured (i.e., detected). As used herein, the term selectively binds  
15 to an antibody refers to the ability of a *Der* HMW-map protein of the present invention to preferentially bind to an antibody, without being able to substantially bind to other antibodies that do not specifically bind to *Der* HMW-map protein. Binding between a *Der* HMW-map protein and an antibody is effected under conditions suitable to form a complex; such conditions (e.g., appropriate concentrations, buffers, temperatures,  
20 reaction times) as well as methods to optimize such conditions are known to those skilled in the art, and examples are disclosed herein. Examples of complex formation conditions are also disclosed in, for example, in Sambrook et al., *ibid*.

As used herein, the term "detecting complex formation" refers to determining if any complex is formed, i.e., assaying for the presence (i.e., existence) of a complex. If  
25 complexes are formed, the amount of complexes formed can, but need not be, determined. Complex formation, or selective binding, between *Der* HMW-map protein and an antibody in the composition can be measured (i.e., detected, determined) using a

30 In one embodiment, a putative antibody-containing composition of the present method includes a biological sample from an animal. A suitable biological sample

includes, but is not limited to, a bodily fluid composition or a cellular composition. A bodily fluid refers to any fluid that can be collected (i.e., obtained) from an animal, examples of which include, but are not limited to, blood, serum, plasma, urine, tears, aqueous humor, cerebrospinal fluid (CSF), saliva, lymph, nasal secretions, milk and feces. Such a composition of the present method can, but need not be, pretreated to remove at least some of the non-IgE or non-IgG isotypes of immunoglobulin and/or other proteins, such as albumin, present in the fluid. Such removal can include, but is not limited to, contacting the bodily fluid with a material, such as the lectin jacalin or an antibody that specifically binds to the constant region of an IgA immunoglobulin (i.e., anti-IgA isotype antibody), to remove IgA antibodies and/or affinity purifying IgE or IgG antibodies from other components of the body fluid by exposing the fluid to, for example, Concanavalin A or protein G, respectively. In another embodiment, a composition includes collected bodily fluid that is pretreated to concentrate immunoglobulin contained in the fluid. For example, immunoglobulin contained in a bodily fluid can be precipitated from other proteins using ammonium sulfate. A preferred composition of the present method is serum.

In another embodiment, an antibody-containing composition of the present method includes a cell that produces IgE or IgG. Such a cell can have IgE or IgG bound to the surface of the cell and/or can secrete IgE or IgG. An example of such a cell includes myeloma cells. IgE or IgG can be bound to the surface of a cell either directly to the membrane of the cell or bound to a molecule (e.g., an antigen) bound to the surface of the cell.

A complex can be detected in a variety of ways including, but not limited to use of one or more of the following assays: an enzyme-linked immunoassay, a radioimmunoassay, a fluorescence immunoassay, a chemiluminescent assay, a lateral flow assay, an agglutination assay, a particulate-based assay (e.g., using particulates such as, but not limited to, magnetic particles or plastic polymers, such as latex or polystyrene beads), an immunoprecipitation assay, a BioCore™ assay (e.g., using colloidal gold) and an immunoblotting assay (e.g., a western blot). Such assays are well known to those skilled in the art. Assays can be used to give qualitative or quantitative results depending on how they are used. Some assays, such as agglutination, particulate

separation, and immunoprecipitation, can be observed visually (e.g., either by eye or by a machines, such as a densitometer or spectrophotometer) without the need for a detectable marker.

In other assays, conjugation (i.e., attachment) of a detectable marker to the *Der* HMW-map protein, to antibody bound to the *Der* HMW-map protein, or to a reagent that selectively binds to the *Der* HMW-map protein or to the antibody bound to the *Der* HMW-map protein (described in more detail below) aids in detecting complex formation. Examples of detectable markers include, but are not limited to, a radioactive label, an enzyme, a fluorescent label, a chemiluminescent label, a chromophoric label or a ligand. A ligand refers to a molecule that binds selectively to another molecule. Preferred detectable markers include, but are not limited to, fluorescein, a radioisotope, a phosphatase (e.g., alkaline phosphatase), biotin, avidin, a peroxidase (e.g., horseradish peroxidase) and biotin-related compounds or avidin-related compounds (e.g., streptavidin or ImmunoPure® NeutrAvidin available from Pierce, Rockford, IL).

In one embodiment, a complex is detected by contacting a putative antibody-containing composition with a *Der* HMW-map protein that is conjugated to a detectable marker. A suitable detectable marker to conjugate to a *Der* HMW-map protein includes, but is not limited to, a radioactive label, a fluorescent label, an enzyme label, a chemiluminescent label, a chromophoric label or a ligand. A detectable marker is conjugated to a *Der* HMW-map protein in such a manner as not to block the ability of the *Der* HMW-map protein to bind to the antibody being detected.

In another embodiment, a *Der* HMW-map protein:antibody complex is detected by contacting a putative antibody-containing composition with a *Der* HMW-map protein and then contacting the complex with an indicator molecule. Suitable indicator molecules of the present invention include molecules that can bind to either the *Der* HMW-map protein or to the antibody bound to the *Der* HMW-map protein. As such, an indicator molecule can comprise, for example, an antigen and an antibody, depending

antibodies, anti-IgG antibodies and antibodies that are known bind to *Der* HMW-map protein but bind to a different epitope on *Der* HMW-map protein than antibodies

identified in the putative antibody-containing composition. Preferred lectins include those lectins that bind to high-mannose groups. An indicator molecule itself can be attached to a detectable marker of the present invention. For example, an antibody can be conjugated to biotin, horseradish peroxidase, alkaline phosphatase or fluorescein.

5           In one preferred embodiment, a *Der* HMW-map protein:antibody complex is detected by contacting the complex with an indicator molecule that selectively binds to an IgE antibody (referred to herein as an anti-IgE reagent) or an IgG antibody (referred to herein as an anti-IgG reagent. Examples of such an anti-IgE or an anti-IgG antibody include, but are not limited to, a secondary antibody that is an anti-isotype antibody (e.g.,  
10   an antibody that selectively binds to the constant region of an IgE or an IgG), an antibody-binding bacterial surface protein (e.g., Protein A or Protein G), an antibody-binding cell (e.g., a B cell, a T cell, a natural killer cell, a polymorphonuclear leukocyte cell, a monocyte cell or a macrophage cell), an antibody-binding eukaryotic cell surface protein (e.g., a Fc receptor), and an antibody-binding complement protein. Preferred  
15   indicator molecules include, but are not limited to, an anti-feline IgE antibody, an anti-feline IgG antibody, an anti-canine IgE antibody, an anti-canine IgG antibody, an anti-human IgE antibody, and an anti-human IgG antibody. As used herein, an anti-IgE or anti-IgG antibody includes not only a complete antibody but also any subunit or portion thereof that is capable of selectively binding to an IgE or IgG heavy chain constant  
20   region. For example, an anti-IgE reagent or anti-IgG reagent can include an Fab fragment or a F(ab')<sub>2</sub> fragment, both of which are described in detail in Janeway et al., in *Immunobiology, the Immune System in Health and Disease*, Garland Publishing, Inc., NY, 1996.

          In another preferred embodiment, a *Der* HMW-map protein:antibody complex is  
25   detected by contacting the complex with an indicator molecule that selectively binds to *Der* HMW-map protein at a different epitope than the epitope at which an antibody in a putative antibody-containing composition binds to *Der* HMW-map protein.

          In one embodiment a complex can be formed and detected in solution. In another embodiment, a complex can be formed in which one or more members of the  
30   complex are immobilized on (e.g., coated onto) a substrate. Immobilization techniques are known to those skilled in the art. Suitable substrate materials include, but are not

limited to, plastic, glass, gel, celluloid, paper, PVDF (poly-vinylidene-fluoride), nylon, nitrocellulose, and particulate materials such as latex, polystyrene, nylon, nitrocellulose, agarose and magnetic resin. Suitable shapes for substrate material include, but are not limited to, a well (e.g., microtiter dish well), a plate, a dipstick, a bead, a lateral flow apparatus, a membrane, a filter, a tube, a dish, a celluloid-type matrix, a magnetic particle, and other particulates. A particularly preferred substrate comprises an ELISA plate, a dipstick, a radioimmunoassay plate, agarose beads, plastic beads, latex beads, immunoblot membranes and immunoblot papers. In one embodiment, a substrate, such as a particulate, can include a detectable marker.

10 A preferred method to detect antibody that binds to *Der* HMW-map protein is an immunoabsorbent assay. An immunoabsorbent assay of the present invention comprises a capture molecule and an indicator molecule. A capture molecule of the present invention binds to an IgE or an IgG in such a manner that the IgE or IgG is immobilized to a substrate. As such, a capture molecule is preferably immobilized to a substrate of  
15 the present invention prior to exposure of the capture molecule to a putative IgE-containing composition or a putative IgG-containing composition. An indicator molecule of the present invention detects the presence of an IgE or an IgG bound to a capture molecule. As such, an indicator molecule preferably is not immobilized to the same substrate as a capture molecule prior to exposure of the capture molecule to a  
20 putative IgE-containing composition or a putative IgG-containing composition.

A preferred immunoabsorbent assay method includes a step of either:

- (a) immobilizing a *Der* HMW-map protein on a substrate prior to contacting a *Der* HMW-map protein with a putative IgE-containing composition or a putative IgG-containing composition to form a *Der* HMW-map protein -immobilized substrate; and
- 25 (b) binding a putative IgE-containing composition or a putative IgG-containing composition on a substrate prior to contacting *Der* HMW-map protein with a putative IgE-containing composition or a putative IgG-containing composition, to form a putative  
30 a *Der* HMW-map protein -immobilized substrate, an anti-IgE antibody immobilized substrate or anti-IgG antibody-immobilized substrate.

- Both a capture molecule and an indicator molecule of the present invention are capable of binding to an IgE, an IgG or *Der* HMW-map protein. Preferably, a capture molecule binds to a different region of an IgE, an IgG or *Der* HMW-map protein than an indicator molecule, thereby allowing a capture molecule to be bound to an IgE, an IgG or
- 5 *Der* HMW-map protein at the same time as an indicator molecule. The use of a reagent as a capture molecule or an indicator molecule depends upon whether the molecule is immobilized to a substrate when the molecule is exposed to an IgE, an IgG or *Der* HMW-map protein. For example, a *Der* HMW-map protein of the present invention is used as a capture molecule when the *Der* HMW-map protein is bound on a substrate.
- 10 Alternatively, a *Der* HMW-map protein is used as an indicator molecule when the *Der* HMW-map protein is not bound on a substrate. Suitable molecules for use as capture molecules or indicator molecules include, but are not limited to, a *Der* HMW-map protein of the present invention, an anti-IgE antibody reagent or an anti-IgG antibody reagent of the present invention.
- 15 An immunoabsorbent assay of the present invention can further comprise one or more layers and/or types of secondary molecules or other binding molecules capable of detecting the presence of an indicator molecule. For example, an untagged (i.e., not conjugated to a detectable marker) secondary antibody that selectively binds to an indicator molecule can be bound to a tagged (i.e., conjugated to a detectable marker)
- 20 tertiary antibody that selectively binds to the secondary antibody. Suitable secondary antibodies, tertiary antibodies and other secondary or tertiary molecules can be selected by those of skill in the art. Preferred secondary molecules of the present invention include an antigen, an anti-IgE idiotype antibody (i.e., an antibody that binds to an epitope unique to the anti-IgE antibody), an anti-IgE isotypic antibody, an anti-IgG
- 25 idiotype antibody (i.e., an antibody that binds to an epitope unique to the anti-IgG antibody), and an anti-IgG isotypic antibody. Preferred tertiary molecules can be selected by a skilled artisan based upon the characteristics of the secondary molecule. The same strategy is applied for subsequent layers.
- In one embodiment, *Der* HMW-map protein is used as a capture molecule by
- 30 being immobilized on a substrate, such as a microtiter dish well or a dipstick. A biological sample collected from an animal is applied to the substrate and incubated

under conditions suitable (i.e., sufficient) to allow for *Der* HMW-map protein:antibody complex formation bound to the substrate (i.e., IgE or IgG in a sample binds to *Der* HMW-map protein immobilized on a substrate). Excess non-bound material (i.e., material from the biological sample that has not bound to the *Der* HMW-map protein), if  
5 any, is removed from the substrate under conditions that retain antigen:antibody complex binding to the substrate. Preferred conditions are generally disclosed in Sambrook et al. *ibid*. An indicator molecule that can selectively bind to an IgE or an IgG bound to the antigen is added to the substrate and incubated to allow formation of a complex between the indicator molecule and the *Der* HMW-map protein:antibody  
10 complex. Excess indicator molecule is removed, a developing agent is added if required, and the substrate is submitted to a detection device for analysis. A preferred indicator molecule for this embodiment is an anti-IgG antibody to detect IgG antibody bound to *Der* HMW-map protein or an anti-IgE antibody to detect IgE antibody bound to *Der* HMW-map protein. Preferably the anti-IgG or anti-IgE antibody are conjugated to  
15 biotin, to a fluorescent label or to an enzyme label.

In one embodiment, an anti-IgE or anti-IgG antibody (e.g., isotype or idiotype specific antibody) is used as a capture molecule by being immobilized on a substrate, such as a microtiter dish well or a dipstick. A biological sample collected from an animal is applied to the substrate and incubated under conditions suitable to allow for  
20 anti-IgE antibody:IgE complex or anti-IgG antibody:IgG complex formation, respectively, bound to the substrate. Excess non-bound material, if any, is removed from the substrate under conditions that retain anti-IgE antibody:IgE complex or anti-IgG antibody:IgG complex binding to the substrate. *Der* HMW-map protein is added to the substrate and incubated to allow formation of a complex between the *Der* HMW-map  
25 protein and the anti-IgE antibody:IgE complex or anti-IgG antibody:IgG complex. Preferably, the *Der* HMW-map protein is conjugated to a detectable marker (preferably to biotin, an enzyme label or a fluorescent label). Excess *Der* HMW-map protein is

30 In one embodiment, an immunosorbent assay of the present invention does not utilize a capture molecule. In this embodiment, a biological sample collected from an

animal is applied to a substrate, such as a microtiter dish well or a dipstick, and incubated under conditions suitable to allow for IgE or IgG binding to the substrate. Any IgE or IgG present in the bodily fluid is immobilized on the substrate. Excess non-bound material, if any, is removed from the substrate under conditions that retain IgE or IgG binding to the substrate. *Der* HMW-map protein is added to the substrate and incubated to allow formation of a complex between the *Der* HMW-map protein and the IgE or IgG. Preferably, the *Der* HMW-map protein is conjugated to a detectable marker (preferably to biotin, an enzyme label or a fluorescent label). Excess *Der* HMW-map protein is removed, a developing agent is added if required, and the substrate is submitted to a detection device for analysis.

Another preferred method to detect IgE or IgG is a lateral flow assay, examples of which are disclosed in U.S. Patent No. 5,424,193, issued June 13, 1995, by Pronovost et al.; U.S. Patent No. 5,415,994, issued May 16, 1995, by Imrich et al; WO 94/29696, published December 22, 1994, by Miller et al.; and WO 94/01775, published January 20, 1994, by Pawlak et al. In one embodiment, a biological sample is placed in a lateral flow apparatus that includes the following components: (a) a support structure defining a flow path; (b) a labeling reagent comprising a bead conjugated to *Der* HMW-map protein, the labeling reagent being impregnated within the support structure in a labeling zone; and (c) a capture reagent comprising an IgE-binding or an IgG-binding composition. The capture reagent is located downstream of the labeling reagent within a capture zone fluidly connected to the labeling zone in such a manner that the labeling reagent can flow from the labeling zone into the capture zone. The support structure comprises a material that does not impede the flow of the beads from the labeling zone to the capture zone. Suitable materials for use as a support structure include ionic (i.e., anionic or cationic) material. Examples of such a material include, but are not limited to, nitrocellulose (NC), PVDF, carboxymethylcellulose (CM). The support structure defines a flow path that is lateral and is divided into zones, namely a labeling zone and a capture zone. The apparatus can further comprise a sample receiving zone located along the flow path, more preferably upstream of the labeling reagent. The flow path in the support structure is created by contacting a portion of the support structure downstream



of the capture zone, preferably at the end of the flow path, to an absorbent capable of absorbing excess liquid from the labeling and capture zones.

In this embodiment, the biological sample is applied to the sample receiving zone which includes a portion of the support structure. The labeling zone receives the sample  
5 from the sample receiving zone which is directed downstream by the flow path. The labeling zone comprises the labeling reagent that binds to IgE or IgG, or both. A preferred labeling reagent is *Der* HMW-map protein conjugated, either directly or through a linker, to a plastic bead substrate, such as to a latex bead. The substrate also includes a detectable marker, preferably a colorimetric marker. Typically, the labeling  
10 reagent is impregnated to the support structure by drying or lyophilization. The sample structure also comprises a capture zone downstream of the labeling zone. The capture zone receives labeling reagent from the labeling zone which is directed downstream by the flow path. The capture zone contains the capture reagent, in this case an anti-IgE or anti-IgG antibody, or both, as disclosed above, that immobilizes the IgE and/or IgG  
15 complexed to the *Der* HMW-map protein in the capture zone. The capture reagent is preferably fixed to the support structure by drying or lyophilizing. The labeling reagent accumulates in the capture zone and the accumulation is assessed visually or by an optical detection device.

In another embodiment, a lateral flow apparatus used to detect IgE or IgG  
20 includes: (a) a support structure defining a flow path; (b) a labeling reagent comprising an anti-IgE or an anti-IgG antibody, or both, as described above, the labeling reagent impregnated within the support structure in a labeling zone; and (c) a capture reagent comprising *Der* HMW-map protein, the capture reagent being located downstream of the labeling reagent within a capture zone fluidly connected to the labeling zone in such a  
25 manner that the labeling reagent can flow from the labeling zone into the capture zone. The apparatus preferably also includes a sample receiving zone located along the flow path, preferably upstream of the labeling reagent. The apparatus preferably also includes  
30 the level of immunocomplex formation using samples of body fluid with the level of immunocomplex formation using control samples. An immunocomplex refers to a

complex comprising an antibody and *Der* HMW-map protein (i.e., *Der* HMW-map protein antibody complex). As such, immunocomplexes form using positive control samples and do not form using negative control samples. As such, if a body fluid sample results in immunocomplex formation greater than or equal to immunocomplex formation using a positive control sample, then the animal from which the fluid was taken is hypersensitive to the *Der* HMW-map protein bound to the substrate. Conversely, if a body fluid sample results in immunocomplex formation similar to immunocomplex formation using a negative control sample, then the animal from which the fluid was taken is not hypersensitive to the *Der* HMW-map protein bound to the substrate

It is within the scope of the present invention that two or more different skin tests and/or *in vitro* tests can be used in combination for diagnostic purposes. For example, the immediate hypersensitivity of an animal to *Der* HMW-map protein can be tested using an *in vitro* immunoabsorbent test capable of detecting IgE antibodies specific for *Der* HMW-map protein in the animal's bodily fluid. While most animals that display delayed hypersensitivity to *Der* HMW-map protein also display immediate hypersensitivity to the allergen, a small number of animals that display delayed hypersensitivity to an allergen do not display immediate hypersensitivity to the allergen. In such cases, following negative results from the IgE-specific *in vitro* test, the delayed hypersensitivity of the animal to *Der* HMW-map protein can be tested using a skin test of the present invention.

The present invention also includes kits to detect antibodies that bind specifically to *Der* HMW-map protein based on each of the disclosed detection methods. One embodiment is a kit to detect *Der* HMW-map protein-specific antibodies comprising *Der* HMW-map protein and a means for detecting an IgE and/or an IgG. Suitable means of detection include compounds disclosed herein that bind to either the *Der* HMW-map protein or to an IgE and/or an IgG. A preferred kit of the present invention further comprises a detection means including an antibody capable of selectively binding to an IgE or IgG disclosed herein and/or a compound capable of binding to a detectable marker conjugated to a *Der* HMW-map protein (e.g., avidin, streptavidin and ImmunoPure® NeutrAvidin when the detectable marker is biotin).

Another preferred kit of the present invention is an allergen kit comprising *Der* HMW-map protein and an allergen commonly detected in the same environment as mite allergen. Suitable and preferred mite-related allergens for use with the present kit include those mite-related allergens disclosed herein.

5 A preferred kit of the present invention includes those in which *Der* HMW-map protein is immobilized on a substrate. If a kit comprises *Der* HMW-map protein and another allergen, the kit can comprise one or more compositions, each composition comprising one allergen. As such, each allergen can be tested separately. A kit can also  
10 herein. Particularly preferred are kits used in a lateral flow assay format. It is within the scope of the present invention that a lateral flow assay kit can include one or more lateral flow assay apparatuses. Multiple lateral flow apparatuses can be attached to each other at one end of each apparatus, thereby creating a fan-like structure.

Another aspect of the present invention includes treating animals susceptible to  
15 or having mite allergy, with a *Der* HMW-map protein formulation of the present invention. According to the present invention, the term treatment can refer to the regulation of a hypersensitive response by an animal to mite allergens. Regulation can include, for example, immunomodulation of cells involved in the animal's hypersensitive response. Immunomodulation can include modulating the activity of molecules typically  
20 involved in an immune response (e.g., antibodies, antigens, major histocompatibility molecules (MHC) and molecules co-reactive with MHC molecules). In particular, immunomodulation refers to modulation of antigen:antibody interactions resulting in inflammatory responses, immunosuppression, and immunotolerization of cells involved in a hypersensitive response. Immunosuppression refers to inhibiting an immune  
25 response by, for example, killing particular cells involved in the immune response. Immunotolerization refers to inhibiting an immune response by anergizing (i.e., diminishing reactivity of a T cell to an antigen) particular cells involved in the immune

includes desensitizing compounds capable of inhibiting an immune response to *Der* HMW-map protein of the present invention. Such desensitizing compounds include

blocking compounds, toleragens and/or suppressor compounds. Blocking compounds comprise compounds capable of modulating antigen:antibody interactions that can result in inflammatory responses, toleragens are compounds capable of immunotolerizing an animal, and suppressor compounds are capable of immunosuppressing an animal. A  
5 desensitizing compound of the present invention can be soluble or membrane-bound. Membrane-bound desensitizing compounds can be associated with biomembranes, including cells, liposomes, planar membranes or micelles. A soluble desensitizing compound of the present invention is useful for: (1) inhibiting a Type I hypersensitivity reaction by blocking IgE:antigen mediated de-granulation of mast cells; (2) inhibiting a  
10 Type III hypersensitivity reaction by blocking IgG:antigen complex formation leading to complement destruction of cells; and (3) inhibiting a Type IV hypersensitivity reaction by blocking T helper cell stimulation of cytokine secretion by macrophages. A membrane-bound desensitizing compound of the present invention is useful for: (1) inhibiting a Type II hypersensitivity reaction by blocking IgG:antigen complex formation  
15 on the surface of cells leading to complement destruction of cells; (2) inhibiting a Type II hypersensitivity reaction by blocking IgG regulated signal transduction in immune cells; and (3) inhibiting a Type IV hypersensitivity reaction by blocking T cytotoxic cell killing of antigen-bearing cells. Examples of desensitizing compounds include, but are not limited to, muteins, mimetopes and antibodies of the present invention, as well as  
20 other inhibitors of the present invention that inhibit binding between a protein of the present invention and IgE.

A desensitizing compound of the present invention can also be covalently linked to a ligand molecule capable of targeting the desensitizing compound to a specific cell involved in a hypersensitive response to *Der* HMW-map protein. Appropriate ligands  
25 with which to link a desensitizing compound include, for example, at least a portion of an immunoglobulin molecule, cytokines, lectins, heterologous allergens, CD8 molecules or major histocompatibility molecules (e.g., MHC class I or MHC class II molecules). Preferred portions of immunoglobulin molecules to link to a desensitizing compound include variable regions capable of binding to immune cell specific surface molecules  
30 and constant regions capable of binding to Fc receptors on immune cells, in particular IgE constant regions. Preferred CD8 molecules include at least the extracellular

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functional domains of the  $\alpha$  chain of CD8. An immune cell refers to a cell involved in an immune response, in particular, cells having MHC class I or MHC class II molecules. Preferred immune cells include antigen presenting cells, T cells and B cells.

In one embodiment, a therapeutic composition of the present invention includes

5 *Der* HMW-map protein of the present invention, a mimetope or mutein thereof, or a *Der* HMW-map nucleic acid molecule of the present invention. Suitable therapeutic compositions of the present invention for treating mite allergy include *Der* HMW-map protein, a mimetope or mutein thereof, or a *Der* HMW-map nucleic acid molecule of the present invention. Preferred therapeutic compositions include: an isolated mite

10 allergenic protein encoded a nucleic acid molecule that hybridizes under stringent hybridization conditions with the complement of a nucleic acid molecule that encodes an amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID

15 NO:13, SEQ ID NO:15, SEQ ID NO:18, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:38, SEQ ID NO:41, and SEQ ID NO:44; a mimetope of the mite allergenic protein; a mutein of the mite allergenic protein; and an isolated nucleic acid molecule selected from the group consisting of: a nucleic acid

20 molecule comprising at least about 150 nucleotides, wherein said nucleic acid molecule comprising at least about 150 nucleotides hybridizes, in a solution comprising 1X SSC and 0% formamide, at a temperature of about 50°C, to a nucleic acid sequence selected from the group consisting of SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:34, SEQ ID NO:36, SEQ ID

25 NO:37, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:43, SEQ ID NO:45, and a nucleic acid sequence encoding a protein comprising the amino acid sequence SEQ ID NO:33 and a complement thereof; and a nucleic acid molecule

30 HMW-map protein, in which a suitable number of cysteine residues have been removed

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or replaced with a non-cysteine residue such that the altered *Der* HMW-map protein is not toxic to an animal (e.g., does not cause anaphylaxis).

In another embodiment, a therapeutic composition of the present invention includes a nucleic acid molecule encoding a *Der* HMW-map protein that can be  
5 administered to an animal in a fashion to enable expression of that nucleic acid molecule into a *Der* HMW-map protein in the animal. Nucleic acid molecules can be delivered to an animal in a variety of methods including, but not limited to, (a) administering a naked (i.e., not packaged in a viral coat or cellular membrane) nucleic acid molecule (e.g., as naked DNA or RNA molecules, such as is taught, for example in Wolff et al., 1990,  
10 *Science* 247, 1465-1468) or (b) administering a nucleic acid molecule packaged as a recombinant virus or as a recombinant cell (i.e., the nucleic acid molecule is delivered by a viral or cellular vehicle).

A naked nucleic acid molecule of the present invention includes a nucleic acid molecule of the present invention and preferably includes a recombinant molecule of the  
15 present invention that preferably is replication, or otherwise amplification, competent. A naked nucleic acid of the present invention can comprise one or more nucleic acid molecules of the present invention in the form of, for example, a bicistronic recombinant molecule having, for example one or more internal ribosome entry sites. Preferred naked nucleic acid molecules include at least a portion of a viral genome (i.e., a viral  
20 vector). Preferred viral vectors include those based on alphaviruses, poxviruses, adenoviruses, herpesviruses, picornaviruses, and retroviruses, with those based on alphaviruses (such as Sindbis or Semliki virus), species-specific herpesviruses and species-specific poxviruses being particularly preferred. Any suitable transcription control sequence can be used, including those disclosed as suitable for protein  
25 production. Particularly preferred transcription control sequence include cytomegalovirus intermediate early (preferably in conjunction with Intron-A), Rous Sarcoma Virus long terminal repeat, and tissue-specific transcription control sequences, as well as transcription control sequences endogenous to viral vectors if viral vectors are used. The incorporation of "strong" poly(A) sequences are also preferred.

30 Naked nucleic acid molecules of the present invention can be administered by a variety of methods. Suitable delivery methods include, for example, intramuscular

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injection, subcutaneous injection, intradermal injection, intradermal scarification, particle bombardment, oral application, and nasal application, with intramuscular injection, intradermal injection, intradermal scarification and particle bombardment being preferred, and intramuscular injection being even more preferred. A preferred  
5 single dose of a naked DNA molecule ranges from about 1 nanogram (ng) to about 1 milligram (mg), depending on the route of administration and/or method of delivery, as can be determined by those skilled in the art. Examples of administration methods are disclosed, for example, in U.S. Patent No. 5,204,253, by Bruner, et al., issued April 20, 1993, PCT Publication No. WO 95/19799, published July 27, 1995, by McCabe, and  
10 PCT Publication No. WO 95/05853, published March 2, 1995, by Carson, et al. Naked DNA molecules of the present invention can be contained in an aqueous excipient (e.g., phosphate buffered saline) and/or with a carrier (e.g., lipid-based vehicles), or it can be bound to microparticles (e.g., gold particles).

A recombinant virus of the present invention includes a recombinant molecule of  
15 the present invention that is packaged in a viral coat and that can be expressed in an animal after administration. Preferably, the recombinant molecule is packaging-deficient and/or encodes an attenuated virus. A number of recombinant viruses can be used, including, but not limited to, those based on alphaviruses, poxviruses, adenoviruses, herpesviruses, picornaviruses and retroviruses. Preferred recombinant  
20 viruses are those based on alphaviruses (such as Sindbis virus), raccoon poxviruses, species-specific herpesviruses and species-specific poxviruses. An example of methods to produce and use alphavirus recombinant virus is disclosed in PCT Publication No. WO 94/17813, by Xiong et al., published August 18, 1994.

When administered to an animal, a recombinant virus of the present invention  
25 infects cells within the recipient animal and directs the production of a protein or RNA nucleic acid molecule that is capable of reducing *Der* HMW-map protein-mediated biological responses in the animal. For example, a recombinant virus comprising a *Der*  
30 reduce *Der* HMW-map protein-mediated biological responses. A preferred single dose of a recombinant virus of the present invention is from about  $1 \times 10^4$  to about  $1 \times 10^7$

virus plaque forming units (pfu) per kilogram body weight of the animal.

Administration protocols are similar to those described herein for protein-based compositions, with subcutaneous, intramuscular, intranasal and oral administration routes being preferred.

- 5           A recombinant cell vaccine of the present invention includes recombinant cells of the present invention that express at least one protein of the present invention. Preferred recombinant cells for this embodiment include *Salmonella*, *E. coli*, *Listeria*, *Mycobacterium*, *S. frugiperda*, yeast, (including *Saccharomyces cerevisiae* and *Pichia pastoris*), BHK, CV-1, myoblast G8, COS (e.g., COS-7), Vero, MDCK and CRFK
- 10 recombinant cells. Recombinant cell vaccines of the present invention can be administered in a variety of ways but have the advantage that they can be administered orally, preferably at doses ranging from about  $10^8$  to about  $10^{12}$  cells per kilogram body weight. Administration protocols are similar to those described herein for protein-based vaccines. Recombinant cell vaccines can comprise whole cells, cells stripped of cell
- 15 walls or cell lysates.

- The efficacy of a therapeutic composition of the present invention to desensitize an animal against mite allergy can be tested in a variety of ways including, but not limited to, using *in vivo* skin test methods disclosed herein, detection of cellular immunity activity in the treated animal, or determine levels of IgE that bind specifically
- 20 to a *Der* HMW-map protein of the present invention. Methods to determine cellular immunity activity and IgE levels in an animal are known to those of skill in the art. In one embodiment, therapeutic compositions can be tested in animal models such as dogs, cats, rabbits and mice, and can also be tested in humans. Such techniques are known to those skilled in the art.

- 25           Preferred nucleic acid molecules to use with a therapeutic composition of the present invention include any *Der* HMW-map nucleic acid molecule disclosed herein, in particular SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:43, SEQ ID NO:45 and/or a
- 30 nucleic acid sequence encoding a protein comprising the amino acid sequence SEQ ID NO:33 and a complement thereof.



A recombinant cell useful in a therapeutic composition of the present invention includes recombinant cells of the present invention that comprises *Der* HMW-map protein of the present invention. Preferred recombinant cells for this embodiment include *Salmonella*, *E. coli*, *Listeria*, *Mycobacterium*, *S. frugiperda*, yeast, (including  
5 *Saccharomyces cerevisiae*), BHK, CV-1, myoblast G8, COS (e.g., COS-7), Vero, MDCK and CRFK recombinant cells. A recombinant cell of the present invention can be administered in a variety of ways but have the advantage that they can be administered orally, preferably at doses ranging from about  $10^8$  to about  $10^{12}$  cells per kilogram body weight. Administration protocols are similar to those described herein  
10 for protein compositions. Recombinant cells can comprise whole cells, cells stripped of cell walls or cell lysates.

One embodiment of the present invention is a method of immunotherapy comprising administering to an animal an effective amount of a therapeutic composition comprising a *Der* HMW-map protein of the present invention. Suitable therapeutic  
15 compositions and methods of administration are disclosed herein. According to the present invention, a therapeutic composition and method of the present invention can be used to prevent or alleviate symptoms associated with mite allergen pathogenesis.

The efficacy of a therapeutic composition of the present invention to effect an allergic response to *Der* HMW-map protein can be tested using standard methods for  
20 detecting *Der* HMW-map protein-mediated immunity including, but not limited to, immediate hypersensitivity, delayed hypersensitivity, antibody-dependent cellular cytotoxicity (ADCC), immune complex activity, mitogenic activity, histamine release assays and other methods such as those described in Janeway et al., *ibid*.

The present invention also includes a therapeutic composition comprising one or  
25 more therapeutic compounds of the present invention. Examples of such therapeutic compounds include, for example, other allergens disclosed herein.

Therapeutic compositions of the present invention can be formulated in an

30 physiologically balanced salt solutions. Nonaqueous vehicles, such as fixed oils, sesame oil, ethyl oleate, or triglycerides may also be used. Other useful formulations include

suspensions containing viscosity enhancing agents, such as sodium carboxymethylcellulose, sorbitol, or dextran. Excipients can also contain minor amounts of additives, such as substances that enhance isotonicity and chemical stability. Examples of buffers include phosphate buffer, bicarbonate buffer and Tris buffer, while  
5 examples of preservatives include thimerosal, o-cresol, formalin and benzyl alcohol. Standard formulations can either be liquid injectables or solids which can be taken up in a suitable liquid as a suspension or solution for injection. Thus, in a non-liquid formulation, the excipient can comprise dextrose, human serum albumin, preservatives, etc., to which sterile water or saline can be added prior to administration.

10 In one embodiment of the present invention, a therapeutic composition can include an adjuvant. Adjuvants are agents that are capable of enhancing the immune response of an animal to a specific antigen. Suitable adjuvants include, but are not limited to, cytokines, chemokines, and compounds that induce the production of cytokines and chemokines (e.g., granulocyte macrophage colony stimulating factor (GM-  
15 CSF), granulocyte colony stimulating factor (G-CSF), macrophage colony stimulating factor (M-CSF), colony stimulating factor (CSF), Flt-3 ligand, erythropoietin (EPO), interleukin 2 (IL-2), interleukin-3 (IL-3), interleukin 4 (IL-4), interleukin 5 (IL-5), interleukin 6 (IL-6), interleukin 7 (IL-7), interleukin 8 (IL-8), interleukin 10 (IL-10), interleukin 12 (IL-12), interferon gamma, interferon gamma inducing factor I (IGIF),  
20 transforming growth factor beta, RANTES (regulated upon activation, normal T cell expressed and presumably secreted), macrophage inflammatory proteins (e.g., MIP-1 alpha and MIP-1 beta), and Leishmania elongation initiating factor (LEIF); bacterial components (e.g., endotoxins, in particular superantigens, exotoxins and cell wall components); aluminum-based salts; calcium-based salts; silica; polynucleotides;  
25 toxoids; serum proteins, viral coat proteins; block copolymer adjuvants (e.g., Hunter's Titermax™ adjuvant (Vaxcel™, Inc. Norcross, GA), Ribi adjuvants (Ribi ImmunoChem Research, Inc., Hamilton, MT); and saponins and their derivatives (e.g., Quil A (Superfos Biosector A/S, Denmark). Protein adjuvants of the present invention can be delivered in the form of the protein themselves or of nucleic acid molecules encoding  
30 such proteins using the methods described herein.

In one embodiment of the present invention, a therapeutic composition can include a carrier. Carriers include compounds that increase the half-life of a therapeutic composition in the treated animal. Suitable carriers include, but are not limited to, polymeric controlled release vehicles, biodegradable implants, liposomes, bacteria, viruses, other cells, oils, esters, and glycols.

One embodiment of the present invention is a controlled release formulation that is capable of slowly releasing a composition of the present invention into an animal. As used herein, a controlled release formulation comprises a composition of the present invention in a controlled release vehicle. Suitable controlled release vehicles include, but are not limited to, biocompatible polymers, other polymeric matrices, capsules, microcapsules, microparticles, bolus preparations, osmotic pumps, diffusion devices, liposomes, lipospheres, and transdermal delivery systems. Other controlled release formulations of the present invention include liquids that, upon administration to an animal, form a solid or a gel *in situ*. Preferred controlled release formulations are biodegradable (i.e., bioerodible).

A preferred controlled release formulation of the present invention is capable of releasing a therapeutic composition of the present invention into the blood of an animal at a constant rate sufficient to attain therapeutic dose levels of the composition to reduce mite allergy in the animal. As used herein, mite allergy refers to cellular responses that occur when mite allergens contact an animal. For example, IgE that specifically binds to mite allergen becomes coupled with Fc epsilon receptor, resulting in Fc epsilon receptor-mediated biological response including release of biological mediators, such as histamine, prostaglandins and/or proteases, that can trigger clinical symptoms of allergy. The therapeutic composition is preferably released over a period of time ranging from about 1 to about 12 months. A preferred controlled release formulation of the present invention is capable of effecting a treatment preferably for at least about 1 month, more preferably for at least about 3 months, even more preferably for at least about 6 months.

Therapeutic compositions of the present invention can be sterilized by conventional methods which do not result in protein degradation (e.g., filtration) and/or lyophilized.

A therapeutic composition of the present invention can be administered to any  
5 animal susceptible to mite allergy as herein described. Acceptable protocols by which to administer therapeutic compositions of the present invention in an effective manner can vary according to individual dose size, number of doses, frequency of dose administration, and mode of administration. Determination of such protocols can be accomplished by those skilled in the art. An effective dose refers to a dose capable of  
10 treating an animal against hypersensitivity to mite allergens. Effective doses can vary depending upon, for example, the therapeutic composition used and the size and type of the recipient animal. Effective doses to immunomodulate an animal against mite allergens include doses administered over time that are capable of alleviating a hypersensitive response by an animal to mite allergens. For example, a first tolerizing  
15 dose can comprise an amount of a therapeutic composition of the present invention that causes a minimal hypersensitive response when administered to a hypersensitive animal. A second tolerizing dose can comprise a greater amount of the same therapeutic composition than the first dose. Effective tolerizing doses can comprise increasing concentrations of the therapeutic composition necessary to tolerize an animal such that  
20 the animal does not have a hypersensitive response to exposure to mite allergens. An effective dose to desensitize an animal can comprise a concentration of a therapeutic composition of the present invention sufficient to block an animal from having a hypersensitive response to exposure to a mite allergen present in the environment of the animal. Effective desensitizing doses can include repeated doses having concentrations  
25 of a therapeutic composition that cause a minimal hypersensitive response when administered to a hypersensitive animal.

A suitable single dose is a dose that is capable of treating an animal against hypersensitivity to mite allergens when administered one or more times over a suitable time period. For example, a preferred single dose of a mite allergen, or mimetope  
30 therapeutic composition is from about 0.5 ng to about 1 g of the therapeutic composition per kilogram body weight of the animal. Further treatments with the therapeutic

composition can be administered from about 1 day to 1 year after the original administration. Further treatments with the therapeutic composition preferably are administered when the animal is no longer protected from hypersensitive responses to mite allergens. Particular administration doses and schedules can be developed by one  
5 of skill in the art based upon the parameters discussed above. Modes of administration can include, but are not limited to, subcutaneous, intradermal, intravenous, nasal, oral, transdermal and intramuscular routes.

A therapeutic composition of the present invention can be used in conjunction with other compounds capable of modifying an animal's hypersensitivity to mite  
10 allergens. For example, an animal can be treated with compounds capable of modifying the function of a cell involved in a hypersensitive response, compounds that reduce allergic reactions, such as by systemic agents or anti-inflammatory agents (e.g., anti-histamines, anti-steroid reagents, anti-inflammatory reagents and reagents that drive immunoglobulin heavy chain class switching from IgE to IgG). Suitable compounds  
15 useful for modifying the function of a cell involved in a hypersensitive response include, but are not limited to, antihistamines, cromolyn sodium, theophylline, cyclosporin A, adrenalin, cortisone, compounds capable of regulating cellular signal transduction, compounds capable of regulating adenosine 3',5'-cyclic phosphate (cAMP) activity, and compounds that block IgE activity, such as peptides from IgE or IgE specific Fc  
20 receptors, antibodies specific for peptides from IgE or IgE-specific Fc receptors, or antibodies capable of blocking binding of IgE to Fc receptors.

Compositions of the present invention can be administered to any animal having or susceptible to mite allergen hypersensitivity. Preferred animals to treat include mammals and birds, with felines, canines, equines, humans and other pets, work and/or  
25 economic food animals. Particularly preferred animals to protect are felines and canines.

Another aspect of the present invention includes a method for prescribing treatment for animals susceptible to or having hypersensitivity to mite allergens, using a

30 an animal at one site an effective amount of a formulation containing a mite allergen of the present invention, or a mimotope thereof (suitable and preferred formulations are

- disclosed herein); (2) intradermally injecting into the animal at a second site an effective amount of a control solution; (3) evaluating if the animal has mite allergen hypersensitivity by measuring and comparing the wheal size resulting from injection of the formulation with the wheal size resulting from injection of the control solution; and
- 5 (4) prescribing a treatment for the mite allergen hypersensitivity.

An alternative preferred method for prescribing treatment for mite allergen hypersensitivity comprises: (1) contacting a first portion of a sample of bodily fluid obtained from an animal to be tested with an effective amount of a formulation containing mite allergen, or a mimetope thereof (suitable and preferred formulations are

10 disclosed herein) to form a first immunocomplex solution; (2) contacting a positive control antibody to form a second immunocomplex solution; (3) evaluating if the animal has mite allergen hypersensitivity by measuring and comparing the amount of immunocomplex formation in the first and second immunocomplex solutions; and (4) prescribing a treatment for the mite allergen hypersensitivity. It is to be noted that

15 similar methods can be used to prescribe treatment for allergies using mite allergen formulations as disclosed herein.

Another aspect of the present invention includes a method for monitoring animals susceptible to or having mite allergen hypersensitivity, using a formulation of the present invention. *In vivo* and *in vitro* tests of the present invention can be used to

20 test animals for mite allergen hypersensitivity prior to and following any treatment for mite allergen hypersensitivity. A preferred method to monitor treatment of mite allergen hypersensitivity (which can also be adapted to monitor treatment of other allergies) comprises: (1) intradermally injecting an animal at one site with an effective amount of a formulation containing mite allergen, or a mimetope thereof (suitable and preferred

25 formulations are disclosed herein); (2) intradermally injecting an effective amount of a control solution into the animal at a second site; and (3) determining if the animal is desensitized to mite allergens by measuring and comparing the wheal size resulting from injection of the formulation with the wheal size resulting from injection of the control solution.

30 An alternative preferred method to monitor treatment of mite allergen hypersensitivity (which can be adapted to monitor treatments of other allergies)

comprises: (1) contacting a first portion of a sample of bodily fluid obtained from an animal to be tested with an effective amount of a formulation containing a mite allergen or mimetope thereof (suitable and preferred formulations are disclosed herein) to form a first immunocomplex solution; (2) contacting a positive control antibody to form a  
5 second immunocomplex solution; and (3) determining if the animal is desensitized to mite allergens by measuring and comparing the amount of immunocomplex formation in the first and second immunocomplex solutions.

The present invention also includes antibodies capable of selectively binding to mite allergen, or mimetope thereof. Such an antibody is herein referred to as an anti-  
10 mite allergen antibody. As used herein, the term "selectively binds to" refers to the ability of such an antibody to preferentially bind to mite allergens and mimetopes thereof. In particular, the present invention includes antibodies capable of selectively binding to *Der* HMW-map protein. Binding can be measured using a variety of methods known to those skilled in the art including immunoblot assays, immunoprecipitation  
15 assays, enzyme immunoassays (e.g., ELISA), radioimmunoassays, immunofluorescent antibody assays and immunoelectron microscopy; see, for example, Sambrook et al., *ibid.*

Antibodies of the present invention can be either polyclonal or monoclonal antibodies. Antibodies of the present invention include functional equivalents such as  
20 antibody fragments and genetically-engineered antibodies, including single chain antibodies, that are capable of selectively binding to at least one of the epitopes of the protein or mimetope used to obtain the antibodies. Preferred antibodies are raised in response to *Der* HMW-map proteins, or mimetopes thereof. More preferred *Der* HMW-map protein against which to raise an antibody includes at least a portion of a protein  
25 having the amino acid sequence SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, and or SEQ ID NO:44, or homologs thereof. Preferably, an

antibody of the present invention has a single site binding affinity of from about  $10^3\text{M}^{-1}$  to about  $10^{12}\text{M}^{-1}$  for a *Der* HMW-map protein of the present invention.

A preferred method to produce antibodies of the present invention includes administering to an animal an effective amount of a *Der* HMW-map protein or  
5 mimotope thereof to produce the antibody and recovering the antibodies. Antibodies raised against defined products or mimetopes can be advantageous because such antibodies are not substantially contaminated with antibodies against other substances that might otherwise cause interference in a diagnostic assay or side effects if used in a therapeutic composition.

10 Antibodies of the present invention have a variety of potential uses that are within the scope of the present invention. For example, such antibodies can be used (a) as vaccines to passively immunize an animal in order to protect the animal from mite allergen hypersensitivity, (b) as positive controls in test kits, and/or (c) as tools to recover desired mite allergens from a mixture of proteins and other contaminants.

15 The following examples are provided for the purposes of illustration and are not intended to limit the scope of the present invention.

### EXAMPLES

It is to be noted that the Examples include a number of molecular biology, microbiology, immunology and biochemistry techniques considered to be known to  
20 those skilled in the art. Disclosure of such techniques can be found, for example, in Sambrook et al., *ibid.*, and related references.

#### Example 1

This example describes the identification of high molecular weight proteins that bind to IgE from dogs known to be allergic to mite allergens.

25 About 5.5 grams (g) of frozen wet *Dermataphagoides farinae* (*Der f*) mites (available from Bayer Allergy, Spokane, WA) were homogenized in a ground glass homogenizer, in either about 30 ml of phosphate buffered saline (PBS) or 0.1 M Tris-HCl, pH 8, each containing complete protease inhibitors (available from Boehringer Mannheim, Indianapolis, IN) to obtain a *Der f* crude extract. The resulting supernatants  
30 were collected and each concentrated in a Centriprep 30 concentrator (available from Amicon, Beverly, MA) by centrifugation at 16,000 x g for about 30 minutes. The



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concentrated supernatants were applied to separate Sephacryl S-100 columns (2.7 x 70 cm; available from Pharmacia, Piscataway, NJ) in PBS or 0.1 M Tris-HCl, pH 8, respectively. The excluded fractions from each column were pooled. Fractions were dialyzed against 10 mM Tris-HCl, pH 8, when PBS was used. The fractions were

5 applied to separate Q-Sepharose columns (2.5 x 5 cm; available from Pharmacia). The Q-Sepharose column was pre-equilibrated in 10 mM Tris-HCl, pH 8, when the fractions containing 0.1 M Tris-HCl, pH 8 were used. Each column was sequentially eluted with about 45 ml of 10 mM Tris-HCl, pH 8, then 0.1 M Tris-HCl, pH 8, then 0.2 M Tris-HCl, pH 8, then 0.3 M Tris-HCl, pH 8, then 0.4 M Tris-HCl, pH 8 and then 0.5 M Tris-HCl,

10 pH 8. Fractions were collected from each elution step. Each fraction was analyzed by western blot for the presence of protein that bound to IgE antibodies present in dog sera isolated from dogs known to be allergic to mite allergens (referred to herein as mite allergic dog antisera or mite allergic antisera). Specifically, proteins contained in the fractions were resolved by 12% Tris-glycine SDS-PAGE and then blotted onto

15 nitrocellulose. The blot was incubated with a pool of sera obtained from dogs known to be allergic to mite allergens, diluted 1:20, using standard buffers. The blot was incubated and then washed using standard procedures. The blot was then incubated with the mouse monoclonal anti-dog IgE antibody DEI38 (1 mg/ml, 1:1000 dilution). The blot was incubated and then washed using standard procedures. The blot was then

20 incubated with donkey anti-mouse IgG antibody conjugated to horseradish peroxidase (1:1000 dilution; available from Jackson Labs, Maine). The presence of HRP-conjugated antibody bound to the blot was detected using standard techniques. An about 70-kD protein was identified in the 0.2 M Tris-HCl, pH 8 fraction, an about 98-kD protein and an about 109-kD protein were identified in the 0.3 M Tris-HCl, pH 8

25 fraction.

The fraction described above that was eluted using 0.3 M Tris-HCl, pH 8 was concentrated in a Centrprep 30 concentrator and then diluted in 20 mM Na-Ac, pH 5.6

30 20 mM Na-Ac, pH 5.6, and then with about 45 ml of a linear gradient from 0 to 0.5 M NaCl in the 20 mM Na-Ac, pH 5.6 buffer at a flow rate of about 1ml/min. Fractions

were collected from the elution procedure and assayed for the presence of high molecular weight proteins using the mite allergic antisera and western blot protocol described above. Fractions containing the high molecular weight proteins were pooled. Trifluoroacetic acid (TFA) was added to a concentration of about 0.05%. The solution  
5 was applied to a TSK-Gel TMS-250 C1 reverse phase column (available from TosohHaas, Montgomeryville, PA) that had been pre-equilibrated in 80% solvent A and 20% solvent B. Solvent A was composed of about 0.05% TFA in water and solvent B was composed of about 0.05% TFA in 90% acetonitrile in water. The column was eluted with about 5 ml of 20% solvent B and then with 36 ml of a linear gradient of  
10 about 20% to about 70% solvent B at 0.6 ml/min. The proteins eluted from the column were resolved by 12% Tris-Glycine PAGE. The gel was stained with Coomassie blue. The stained gel is shown in Fig. 1. Lane 1 contains Mark-12 protein molecular weight markers (available from Novex, San Diego, CA), lane 2 contains the protein eluted from the reverse phase column, and lane 3 contains SeeBlue™ protein molecular weight  
15 markers (available from Novex). Two major proteins were identified in the eluant. The molecular weights of the proteins were determined using a BioRad™ Multi-Analyst™/PC Image System (available from BioRad Corp.). The higher molecular weight protein in lane 2 of Fig. 1 was determined to be about 109 kD, referred to herein as mite allergen protein A (mapA). The lower molecular weight protein in lane 2 of Fig.  
20 1 was determined to be about 98 kD, referred to herein as mite allergen protein B (mapB). The purity of the combined proteins was greater than 85% purity, i.e., less than 15% impurities. This purified eluant is referred to herein as the *D. farinae* high molecular weight map (HMW-map) composition.

#### Example 2.

25 This example describes N-terminal sequencing of proteins in the *D. farinae* HMW-map composition.

Proteins contained in the 0.3 M Tris-HCl, pH 8 fraction obtained as described above in Example 1 were resolved by SDS-PAGE using a 12% Tris-glycine polyacrylamide-SDS gel, followed by coomassie staining. The proteins were blotted onto  
30 PVDF, stained with Coomassie R-250 and destained using standard procedures. The proteins corresponding to the about 98 kD and about 109 kD bands were excised and

subjected separately to N-terminal amino acid sequencing using techniques known to those skilled in the art. A partial N-terminal amino acid sequence of about 14 amino acids was deduced for both proteins and the sequences were determined to be identical. The N-terminal amino acid sequence is represented herein as SEQ ID NO:1, having the  
5 amino acid sequence: Ser Ile Lys Arg Asp His Asn Asp Tyr Ser Lys Asn Pro Met.

The proteins in the *D. farinae* HMW-map composition were also submitted to proteolytic cleavage in order to obtain internal amino acid sequence data. Specifically, the *D. farinae* HMW-map composition was cleaved with Endoproteinase Asp-N (available from Boehringer Mannheim Biochemica, Indianapolis, IN) using methods  
10 standard in the art. The digested protein was then resolved by HPLC using the method described by Stone et al., Enzymatic Digestion of Proteins and HPLC Peptide Isolation, in A Practical Guide to Protein and Peptide Purification for Microsequencing, PT Matsudaira ed., Academic Press, San Diego, CA. Twelve proteolytic fragments were isolated, that are referred to herein as map(1), map(2), map(3), map(4), map(5), map(6),  
15 map(7), map(8), map(9), map(10), map(11) and map(12).

The N-terminal partial amino acid sequence of map(1) was determined to be Asp Tyr Glu Tyr Pro Gly Ser Arg Leu Gly Asn Pro Lys Ala Pro Leu Tyr Lys Arg Pro, also denoted SEQ ID NO:2. The N-terminal partial amino acid sequence of map(2) was determined to be Asp Ile Pro His Pro Thr Asn Ile His Lys Tyr Leu Val Cys Glu Ser Val  
20 Asn Gly Gly, also denoted SEQ ID NO:3. The N-terminal partial amino acid sequence of map(3) was determined to be Asp Pro Ala Lys Gly Met Ser Pro Pro Gly Phe Ile Val Gly Glu Glu Gly Val Leu Ser, also denoted SEQ ID NO:4. The N-terminal partial amino acid sequence of map(4) was determined to be Asp Glu Lys Asn Ser Phe Glu Cys Ile Leu Gly Pro, also denoted SEQ ID NO:5. The N-terminal partial amino acid  
25 sequence of map(5) was determined to be Asp Ala Phe Glu Pro His Gly Tyr Leu Leu Thr Ala Ala Val Ser Pro Gly Lys, also denoted SEQ ID NO:6. The N-terminal partial amino acid sequence of map(6) was determined to be Asp Lys Gln Asn Tyr Leu Ala Leu Val  
30 Gln Phe Leu Gln Ser Val Leu Asn Asn Gly Ala Thr Arg Gln, also denoted SEQ ID NO:7. The N-terminal partial amino acid sequence of map(8) was determined to be Asp Glu

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Xaa Asn Val Met Xaa Tyr Val Leu Tyr Thr Met His Tyr Tyr Leu Asn Asn Gly Ala Thr Arg, also denoted SEQ ID NO:9, in which Xaa represents any amino acid. The N-terminal partial amino acid sequence of map(9) was determined to be Asp Lys Leu Val Met Gly Val Pro Phe Tyr Gly Arg Ala Xaa Ser Ile Glu, also denoted SEQ ID NO:10, in which Xaa represents any amino acid. The N-terminal partial amino acid sequence of map(10) was determined to be Asp Ile Pro His Pro Thr Asn Ile His Lys Tyr Leu Val Cys Glu Ser Val Asn Gly, also denoted SEQ ID NO:11. The N-terminal partial amino acid sequence of map(11) was determined to be Asp Tyr Ala Lys Asn Pro Lys Arg Ile Val Cys Ile Val Gly Thr Glu Gly Val Leu Ser, also denoted SEQ ID NO:12. The N-terminal partial amino acid sequence of map(12) was determined to be Asp Pro Ala Lys Gly Met Ser Pro Pro Gly He Ile Val Gly Glu Glu Gly Val Leu Ser, also denoted SEQ ID NO:13. Since the amino acid sequences for map(1), map(2), map(3), map(4), map(5), map(6), map(7), map(8), map(9), map(10), map(11), map(12), and map(13) were generated from a mixture of mapA and mapB proteins, these sequences do not necessarily represent partial sequences of a single protein.

### Example 3

This example describes the purification of a 70-kD protein that binds to IgE from dogs known to be allergic to mite allergens.

The fraction described above in Example 1 that was eluted using 0.2 M Tris-HCl, pH 8 was concentrated in a Centriprep 30 concentrator and then diluted in 20 mM Na-Ac, pH 5.6. The diluted protein was then applied to a PolyCat A HPLC cation exchange column. The column was eluted with about 10 ml of 20 mM Na-Ac, pH 5.6, and then with about 45 ml of a linear gradient from 0 to 0.5 M NaCl in the 20 mM Na-Ac, pH 5.6 buffer at a flow rate of about 1 ml/min. Fractions were collected from the elution procedure and assayed for the presence of 70-kD protein using the mite allergic antisera and western blot protocol described above. Fractions containing the 70-kD protein were pooled. Trifluoroacetic acid (TFA) was added to a concentration of about 0.05%. The solution was applied to a TSK-Gel TMS-250 C1 reverse phase column that had been pre-equilibrated in 80% solvent A and 20% solvent B. Solvent A was composed of about 0.05% TFA in water and solvent B was composed of about 0.05% TFA in 90% acetonitrile in water. The column was eluted with about 3 ml of

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20% solvent B and then with 36 ml of a linear gradient of about 20% to about 70% solvent B at 0.6 ml/min. An about 70-kD protein of >90% purity was obtained. The about 70-kD protein is referred to herein as mapC.

5 N-terminal sequence of a region on an SDS-PAGE corresponding to the 70 kD protein (mapC) was obtained as described in Example 2. An N-terminal amino acid sequence of about 21 amino acids was deduced with an 80% confidence level, and is represented herein as SEQ ID NO:33, having the following amino acid sequence: Gln Ser Arg Asp Arg Asn Asp Lys Pro Tyr Xaa Ile Val Lys Lys Lys Lys Lys Ala Leu Asp.

#### Example 4

10 This example describes the binding of the *D. farinae* HMW-map composition (i.e., containing mapA and mapB) to canine IgE in dog sera isolated from dogs known to be allergic to mite allergens.

Multiple wells of an Immulon II microtiter plate were coated with about 100 nanograms per well (ng/well) of a *D. farinae* HMW-map composition isolated according to the method described above in Example 1, diluted in CBC buffer. The plate was incubated overnight at 4°C. Following incubation, the *D. farinae* HMW-map composition-containing solution was removed from the plate, and the plate was blotted dry. The plate was then blocked using about 200  $\mu$ l/well of 4.0% fetal calf serum contained in phosphate buffered saline (PBS) having 0.05% Tween-20 (PBSTFCS) for about 1 hour at room temperature. The plate was then washed four times with 0.05% Tween-20 in PBS (PBST) using an automatic washer (available from Dynatech, Chantilly, VA). About 100  $\mu$ l/well of a 1:10 dilution in PBSTFCS of serum samples isolated from different dogs known to be sensitive to mite allergens in intradermal skin tests were added to the plate. A negative control group of sera was also added to the plate comprising a combination of sera from six dogs that were raised in a barrier facility (available from Harlan Bioproducts, Indianapolis, IN). Some wells did not receive dog sera so that background binding levels could be determined. The plate was incubated for

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30 (produced as described in Frank et al., WO 98 23964, published November 24, 1997) contained in PBSTFCS was added. The plate was incubated for about 1 hour at room

temperature and then washed four times with PBST. About 100  $\mu$ l of about 0.25  $\mu$ g/ml streptavidin conjugated to horseradish peroxidase (available from Kirkegaard and Perry Laboratories (KPL), Gaithersburg, MD; diluted in PBST) was added to each well that received experimental or control samples. The plates were then incubated for about 1  
5 hour at room temperature and washed four times with PBST. About 100  $\mu$ l of TMB substrate (available from KPL), that had been pre-warmed to room temperature, was added to each well. The plate was then incubated for about 10 minutes at room temperature and then about 100  $\mu$ l/well of Stop Solution (available from KPL) was added. Optical densities (O.D.) of wells were read on a Spectramax Microtiter Plate  
10 (available from Molecular Devices Inc.) reader at 450 nm within 10 minutes of adding the stop solution.

The O.D. readings obtained using the negative control sample and the background wells were 0 O.D. Sera from 5 of 26 mite allergen sensitive dogs generated O.D. readings between about 2,000 O.D. and about 3,200 O.D. Sera from 3 other mite  
15 allergen sensitive dogs generated O.D. readings between about 1,000 O.D. and 2,000 O.D. Sera from 3 other mite allergen sensitive dogs generated O.D. readings between about 500 O.D. and 1,000 O.D. Sera from 7 other mite allergen sensitive dogs generated O.D. readings between about 200 O.D. and 500 O.D. Sera from 6 other mite allergen sensitive dogs generated O.D. readings less than 50 O.D. Thus, the results indicate that  
20 sera from dogs known to be sensitive to mite allergens contain IgE antibodies that bind specifically to the mapA and mapB proteins of the present invention.

#### Example 5

This example describes the binding of the 70-kD *D. farinae* protein to canine IgE in dog sera isolated from dogs known to be allergic to mite allergens.

25 Multiple wells of an Immulon II microtiter plate were coated with about 100 ng/well of 70-kD *D. farinae* protein (referred to herein as mapC) isolated according to the method described above in Examples 1 and 3, diluted in CBC buffer. The plate was incubated overnight at 4°C. The plate was blocked and washed using the method described in Example 4. About 100  $\mu$ l/well of a 1:10 dilution in PBSTFCS of serum  
30 samples isolated from different dogs known to be sensitive to mite allergens in intradermal skin tests were added to the plate. Negative control samples were also

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added to the plate comprising SPF serum samples (serum from dogs maintained in a barrier facility and therefore never exposed to mite allergens). Some wells did not receive dog sera so that background binding levels could be determined. The plate was incubated for about 1 hour at room temperature and then washed four times with PBST.

- 5 Biotinylated human FcεR alpha chain protein was then added and the presence of IgE bound to the plate was detected using the methods described in Example 4.

The O.D. readings obtained using the negative control sample and the background wells were 0 O.D. Sera from 3 of 26 mite allergen sensitive dogs generated O.D. readings between about 1,500 O.D. and about 2,700 O.D. Sera from 5 other mite  
10 allergen sensitive dogs generated O.D. readings between about 800 and about 1,500 O.D. Sera from 4 other mite allergen sensitive dogs generated O.D. readings between about 500 O.D. and about 800 O.D. Sera from 6 other mite allergen sensitive dogs generated O.D. readings between about 200 O.D. and 500 O.D. Sera from 8 other mite allergen sensitive dogs generated O.D. readings less than 50 O.D. Thus, the results  
15 indicate that sera from dogs known to be sensitive to mite allergens contain IgE antibodies that bind specifically to the mapC protein of the present invention.

#### Example 6

This example describes the binding of mapA, mapB or mapC proteins to feline IgE in cat sera isolated from cats shown by *in vitro* testing to be hypersensitive to mite  
20 allergens.

Multiple wells of an Immulon II microtiter plate were coated with about 100 ng/well of a *D. farinae* HMW-map composition (isolated according to the method described above in Example 1) and 70-kD *D. farinae* protein (isolated according to the method described above in Example 3). Other wells of the plate were coated with 400  
25 ng/well of whole *Dermatophagoides pteronyssius* extract (available from Greer Laboratories, Inc., Lenoir, NC; concentrated 8-fold prior to use) or whole *D. farinae* extract (available from Miles, Inc., Elkhart, IN). All samples were diluted in CBC

in PBST. The S of serum samples isolated from different cats known to be sensitive to mite allergens in *in vitro* allergen testing were added to the plate. Sera from seven control

cats (#15, #16, #17, #18, #19, #20, and #21), shown not to be sensitive by *in vitro* test to dust mite allergens, were also tested. Some wells did not receive cat sera so that background binding levels could be determined. The plate was incubated for about 1 hour at room temperature and then washed four times with PBST. Biotinylated human FcεR alpha chain protein was then added and the presence of IgE bound to the plate was detected using the methods described in Example 4.

The results are shown below in Table 1. All values represent O.D. values times 1,000. HDM refers to cats that are sensitive to house dust mite allergens (by serological test, i.e. an ELISA to whole *D. farinae* extract).

Table 1.

Cat #	HDM	Whole <i>Der p</i>	Whole <i>Der f</i>	mapA and mapB	mapC
1	+	54	173	211	400
2	+	437	454	245	352
3	+	96	88	17	36
4	+	35	179	278	758
5	+	123	23	0	0
6	+	2	10	0	0
7	+	84	321	439	445
8	+	125	333	611	599
9	+	2459	2737	1613	507
10	+	17	0	0	0
11	+	146	347	243	586
12	+	31	100	102	223
13	+	56	171	267	292
14	+	121	146	163	185
15	-	0	0	0	8
16	-	0	0	0	0
17	-	0	0	0	0
18	-	0	0	0	0
19	-	0	0	0	0
20	-	0	0	0	0
21	-	23	0	0	0

The results indicate that sera from some of the cats known to be sensitive to mite allergens contain IgE antibodies that bound specifically to the mapA, mapB or mapC proteins of the present invention. In addition, some sera containing IgE that bound to the mapA, mapB or mapC proteins also contain IgE antibodies that bound to whole *D.*



*pteronysius* extract. The control sera did not contain IgE antibodies that bound to either the mapA, mapB or mapC proteins of the present invention.

#### Example 7

This example demonstrates the ability of the *D. farinae* HMW-map composition  
5 to induce a hypersensitive response in dogs.

To determine whether the *D. farinae* HMW-map composition described in  
Example 1 was capable of inducing an allergic response in animals susceptible to dust  
mite allergic responses, skin tests were performed on dogs that actively demonstrate  
clinical signs for dust mite allergy (referred to herein as atopic dogs). Normal dogs  
10 include dogs that do not show symptoms of mite allergy but may be susceptible to a mite  
allergic response. Each dog (i.e., 4 normal and 4 atopic dogs) was shaved in the lateral  
thorax/abdominal area and intradermally injected in different sites in that area with an  
about 1:50,000 dilution of *D. farinae* crude extract isolated by the method described in  
Example 1, with about 2  $\mu$ g of the purified *D. farinae* HMW-map composition and/or  
15 with control solutions, i.e., saline, as a negative control, and a 1:1000 dilution of  
histamine as a positive control. All four normal dogs and all 4 atopic dogs received *D.*  
*farinae* whole extract. Three of the normal dogs and 2 of the atopic dogs received the *D.*  
*farinae* HMW-map composition. All 8 of the dogs received both the negative and  
positive control samples. The total volume per injection was 50 microliters ( $\mu$ l), with  
20 the compositions and controls being diluted in saline. The injections were administered  
as single injections.

All injection sites were objectively measured in millimeters (mm) at 15 minutes  
and scored either (+) or (-) when compared with the control samples. The subjective  
scoring was performed by Andrew Hillier, D.V.M., at Ohio State University, Columbus.  
25 OH. The results are shown in Table 2:

Table 2

	Normal Dog 1	Normal Dog 2	Normal Dog 3	Normal Dog 4	Atopic Dog 1	Atopic Dog 2	Atopic Dog 3	Atopic Dog 4
Whole Extract	+	+	+	-	+	+	-	-
HMW-map	+	+	-	n/a	+	-	n/a	n/a
Neg. Control	-	-	-	-	-	-	-	-
Histamine	+	+	+	+	+	+	+	+

n/a = not applicable

The results indicate that the *D. farinae* HMW-map composition was capable of inducing an immediate hypersensitive response in dogs including atopic dogs. Thus, the HMW-map composition is sufficiently allergenic to induce a hypersensitive response in dogs including atopic dogs.

Table 3 describes the results of the following experiment. IgE to the HMW-map composition was measured in the serum of three groups of dogs: *D. farinae* allergic (HDM-AD), atopic (to other allergens) but not HDM allergic (AD), and naive dogs using ELISA. These dogs were also tested by intradermal skin test to *D. farinae* whole extract and to the HMW-map composition.

Table 3. Skin test and ELISA data for *D. farinae* whole extract and for HMW-map composition in *D. farinae*-allergic, atopic but not HDM-allergic, and naive dogs.

Dog	Clinical status	Df IDST 1:50,000	Df ELISA	HMW-map IDST Iug	HMW-map ELISA
1	HDM-AD	+	1968	+	2876
2	HDM-AD	+	407	-	954
3	HDM-AD	+	3921	+	3465
4	HDM-AD	+	153	+	198
5	HDM-AD	+	1712	+	997
6	HDM-AD	+	1833	+	2006
7	HDM-AD	+	4200	+	4200
8	HDM-AD	+	2851	+	3559
9	HDM-AD	+	122	+	209
10	HDM-AD	+	1627	+	566
11	HDM-AD	+	1185	+	1307
12	HDM-AD	+	308	+	101
13	HDM-AD	+	341	+	433
14	AD	-	1	-	0
15	AD	-	8	-	2
16	AD	ND	66	ND	87
17	Normal	-	24	-	40
18	Normal	-	53	ND	369
19	Normal	-	37	-	21
20	SPF beagle	ND	0	ND	0
21	SPF beagle	ND	6	ND	1

All dogs that were positive by ELISA for whole *D. farinae* extract were also positive for the HMW-map composition allergen. Of the eight dogs that were ELISA negative for whole *D. farinae* extract, 7 of 8 were also negative for the HMW-map composition.

#### Example 8

This example describes the isolation of nucleic acid molecules encoding a *Der* HMW-map composition of the present invention.

*Der* HMW-map composition nucleic acid molecules were identified and isolated

A *D. farinae* cDNA library was prepared as follows. Total

RNA was extracted from about 2 grams of flash frozen and pulverized house dust mites.

using an acid-guanidinium-phenol-chloroform method similar to that described by Chomzynski et al., 1987, *Anal. Biochem.* 162,156-159. Poly A<sup>+</sup> selected RNA was separated from the total RNA preparation by oligo-dT cellulose chromatography using the mRNA Purification Kit (available from Pharmacia Biotech, Newark, NJ), according to the method recommended by the manufacturer. A cDNA library was constructed in lambda-Uni-ZAP<sup>TM</sup> XR vector (available from Stratagene), using Stratagene's ZAP-cDNA Synthesis Kit protocol. Approximately 5 µg of Poly A<sup>+</sup> RNA was used to produce the *Dermatophadoides farinae* cDNA library.

B. Preparation of PCR primers.

Further N-terminal amino acid sequence analysis was performed according to the methods described above in Example 2. A partial N-terminal amino acid sequence of 34 amino acids was deduced and is represented by SEQ ID NO:24, having the amino acid sequence: Ser Ile Lys Arg Asp His Asn Asp Tyr Ser Lys Asn Pro Met Met Ile Val Xaa Tyr Tyr Gly Gly Ser Ser Gly Tyr Gln Ser Xaa Lys Arg Xaa Xaa Thr (wherein "Xaa" represents any amino acid residue). The amino acid sequences of SEQ ID NO:4 (described above in Example 2) and SEQ ID NO:24 were used to design synthetic oligonucleotide primers. Sense primer Derf1 derived from SEQ ID NO:24, having the nucleotide sequence 5' AAA CGT GAT CAT AAY GAT TAY TCN AAR AAY C 3' (wherein Y represents C or T, R represents A or G, and N represents A, C, T or G), designated SEQ ID NO: 25 or sense primer Derf2, derived from SEQ ID NO:24, having the nucleotide sequence 5' AAA CGT GAT CAT AAY GAT TAY AGY AAR AAY C 3', designated SEQ ID NO:26, were used in combination with antisense primer Derf3 derived from SEQ ID NO:4, having the nucleotide sequence 5' CCT TCT TCA CCN ACR ATC AAN CC 3', denoted SEQ ID NO:27, or antisense primer Derf4 derived from SEQ ID NO:4, having the nucleotide sequence 5' CCT TCT TCA CCN ACR ATG AAN CC 3', denoted SEQ ID NO:28.

The foregoing primers were then used to screen the *Der f* cDNA library using standard polymerase chain reaction amplification (PCR) techniques. All attempts to identify a cDNA that hybridized to the primers failed.

C. Immunoscreening the *D. farinae* cDNA library using anti-*Der* HMW-mapcomposition antibodies.

Since attempts to isolate a cDNA clone using PCR methods failed, the inventors screened the *D. farinae* cDNA library using an antiserum produced as follows. Protein isolated according to the method described above in Example 1 was used as a source of antigen to generate rabbit polyclonal antibodies, referred to herein as anti-*Der* HMW-map composition antibodies. The preparation of rabbit polyclonal antibodies was

5 carried out using standard techniques.

About 7.5 ml of *Escherichia coli* (XL1 Blue, O.D.<sub>600</sub>=0.5) was incubated with  $3.0 \times 10^4$  pfu of phage from a *Dermatophagoides farinae* ZAP-cDNA library ( $1.8 \times 10^9$  pfu/ml), at 37°C for 15 min and plated in 30 ml Luria-Bertani (LB) medium agar plates

10 (150 mm). The plates were incubated at 37°C over night. Each plate was then overlaid with an IPTG (10 mM) treated nitrocellulose filter for about 4 hours at 37°C. The filters were then removed and washed with Tris buffered saline (pH 7.5) containing 0.1% Tween (TBST), for 5 minutes. The filters were blocked with a solution of 1% dried powder milk, 1% BSA, 2% goat serum and 0.15% gelatin, prepared in TBST, for 2 hours

15 at room temperature. Filters were then incubated with the anti-*Der* HMW-map composition antibodies at a dilution of 1:1000, contained in the above blocking solution at 4°C, overnight. The mixture was then incubated with a donkey anti-rabbit IgG antibody conjugated to horseradish peroxidase (available from Jackson ImmunoResearch, West Grove, PN) for 2 hours at room temperature. All of the filters

20 were washed with blocking solution contained in TBST (3 x 15 min/wash) between each incubation. All of the filters were then treated to a final wash in Tris buffered saline (pH 7.5) for 5 minutes at room temperature. Immunocomplexed plaques were identified by immersing the filters into the developing solution (TMB Peroxidase Substrate/TMB Peroxidase Solution/TMB Membrane Enhancer from Kirkegaard & Perry Laboratories)

25 at 1/1/0.1 volume ratio to produce a color reaction. One hundred and twenty three plaques were identified and 50 plaques were further plaque purified two more times under the same immunoscreening condition as described above.

Further analyzed by PCR amplification using the primers described above in section 8B DNA from the 50 plaques was amplified using a mixture of the 4 primers identified by

SEQ ID NO: 25, SEQ ID NO:26, SEQ ID NO:27 and SEQ ID NO:28. PCR amplification was conducted using standard techniques. One resulting PCR amplification product comprised a fragment of about 700 nucleotides. The PCR product was cloned into the InVitrogen, Corp., TA™ cloning vector (procedures provided by InVitrogen, Corp.) and subjected to DNA sequence analysis using standard techniques. The phagemid from the purified phage that were determined to contain sequences encoded in the 700-bp PCR product were rescued and subjected to DNA sequence analysis using standard techniques.

A clone was isolated that included about a 1752-nucleotide insert, referred to herein as nDorf98<sub>1752</sub>. Nucleic acid sequence was obtained using standard techniques from nDorf98<sub>1752</sub>, to yield a *Dermatophagoides farinae* nucleic acid molecule named nDorf98<sub>1752</sub> composed of a coding strand having nucleic acid sequence SEQ ID NO:14 and a complementary strand having a nucleic acid sequence SEQ ID NO:16. Translation of SEQ ID NO:14 suggests that nucleic acid molecule nDorf98<sub>1752</sub> encodes a full-length flea protein of about 555 amino acids, referred to herein as PDorf98<sub>555</sub>, having amino acid sequence SEQ ID NO:15, assuming an open reading frame in which the first codon spans from nucleotide 1 through nucleotide 3 of SEQ ID NO:14 and a stop codon spanning from nucleotide 1666 through nucleotide 1668 of SEQ ID NO:14. The amino acid sequence of PDorf98<sub>555</sub> is encoded by the nucleic acid molecule nDorf98<sub>1665</sub>, having a coding strand with the nucleic acid sequence SEQ ID NO:17 and a complementary strand with the nucleic acid sequence SEQ ID NO:19. PDorf98<sub>555</sub>, also represented by SEQ ID NO:18, has an estimated molecular weight of about 63.2 kD and an estimated pI of about 5.33. Analysis of SEQ ID NO:15 suggests the presence of a signal peptide spanning from about amino acid 1 through about amino acid 19. The proposed mature protein, denoted herein as PDorf<sub>536</sub>, contains about 536 amino acids, the sequence of which is represented herein as SEQ ID NO:21, and is encoded by a nucleic acid molecule referred to herein as nDorf98<sub>1608</sub>, represented by SEQ ID NO:20, the coding strand, and SEQ ID NO:22, the complementary strand. The amino acid sequence of flea PDorf98<sub>536</sub> (i.e. SEQ ID NO:21) predicts that PDorf98<sub>536</sub> has an estimated molecular weight of 61.2 kD, and an estimated pI of about 5.26.

Comparison of amino acid sequence SEQ ID NO:15 with amino acid sequences reported in GenBank indicates that SEQ ID NO:15 showed the most homology, i.e., about 42% identity, with a chitinase protein from *Anopheles gambiae* (GenBank accession number 2654602). Comparison of nucleic acid sequence SEQ ID NO:17 with  
5 nucleic acid sequences reported in GenBank indicates that SEQ ID NO:17 showed the most homology, i.e., about 58% identity between SEQ ID NO:17 and *Chelonus sp.* venom chitinase mRNA (GenBank accession number U10422).

#### Example 9

This example describes the purification of a 60-kD protein that binds to IgE from  
10 dogs known to be allergic to mite allergens and partial amino acid sequences derived from this 60-kD protein.

##### A. Purification of a 60 kD protein

*D. farinae* extract was prepared and fractionated on a Sephacryl S-100 column according to the methods described above in Example 1. Fractions were collected from  
15 the Sephacryl S-100 column after the excluded peak (fractions 29 through 35) and were pooled. The pooled fractions were then diluted 1:1 with 10 mM Tris-HCl, pH 8, and applied to a Q-sepharose column and fractions obtained using the methods described above in Example 1. The fraction that eluted in 0.4 M Tris-HCl was concentrated and further purified through a TMS 250 reverse phase HPLC column using the methods  
20 described above in Example 1. The proteins in the fractions were resolved by 14% Tris-glycine SDS-PAGE using similar methods described for resolution of proteins on the 12% gel in Example 1. The stained gel is shown in Fig. 2. A protein was identified having a molecular weight of about 60 kD (Fig. 2, lane 4) of about 90% purity that eluted at about 50% B (.05%TA in 90% acetonitrile). The molecular weight of the  
25 denoted 60-kd protein was estimated to be 56.11 kd using the BioRad Multi-Analyst/PC Version 1.1 program and Mark-12 protein molecular weight markers. The about 60-kd protein is referred to herein as mapD protein.

stained with Coomassie R-250 and destained using standard procedures. The protein corresponding to the about 60-kd band was excised and subjected to N-terminal amino

acid sequencing using techniques known to those skilled in the art. A partial N-terminal amino acid sequence of about 25 amino acids was deduced for the protein and the amino acid sequence, represented herein as SEQ ID NO:23, was determined to be: Xaa Leu Glu Pro Lys Thr Val Cys Tyr Tyr Glu Ser Trp Val His His Arg Gln Gly Glu Gly Lys  
5 Met Asp Pro (wherein Xaa refers to any amino acid).

The protein corresponding to the 60 kd region was also submitted to proteolytic cleavage in order to obtain internal amino acid sequence data. Digestion of the 60-kd protein and reverse-phase chromatography were carried out as described in Example 1. Four proteolytic fragments were isolated and sequenced, and are referred to herein as  
10 map(13), map(14), map(15), and map(16).

The N-terminal partial amino acid sequence of map(13) was determined to be Gln Tyr Gly Val Thr Gln Ala Val Val Thr Gln ProAla, also denoted SEQ ID NO:29. The N-terminal partial amino acid sequence of map(14) was determined to be Asp Glu Leu Leu Met Lys Ser Gly Pro Gly Pro, also denoted SEQ ID NO:30. The N-terminal  
15 partial amino acid sequence of map(15) was determined to be Asp Met Glu His Phe Thr Gln His Lys Gly Asn Ala Lys Ala Met Ile Ala Val Gly Gly Ser Thr Met Ser, also denoted SEQ ID NO:31. The N-terminal partial amino acid sequence of map(16) was determined to be Asp Ala Asn Glu Glu Ala Arg Ser Gln Leu Pro Glu Thr Ala Met Val Leu Ile Lys Ser Gln, denoted SEQ ID NO:32.

20 Example 10.

This example describes the isolation and sequencing of nucleic acid molecules encoding a portion of the *D. farinae* 60 kD (mapD) allergen.

A *D. farinae* library was prepared as described previously in Example 8. A degenerate synthetic oligonucleotide primer was designed from the N-terminal amino  
25 acid sequence deduced for *D. farinae* 60 kD-protein (SEQ ID NO:23): Primer 1, a sense primer corresponding to amino acid residues from about 3 through about 11 of SEQ ID NO:23 has the sequence 5' GAACCAAAA CHGTNTGYTA YTAYG 3', also known as SEQ ID NO:46, where H represents A or C or T, N represents A or C or G or T, and Y represents C or T. PCR amplification of fragments from the *D. farinae* library was  
30 conducted using standard techniques. A PCR amplification product was generated using



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a combination of SEQ ID NO:46 (primer 1) and the M13 forward universal primer 5'GTAAAACGACG GCCAGT 3', denoted SEQ ID NO:47.

A second, nested PCR reaction was carried out on the products of the first PCR reaction. A synthetic oligonucleotide was synthesized that corresponded to a region  
5 spanning from about amino acid residue 1 through amino acid residue 10 of the 60-kD protein internal amino acid sequence, SEQ ID NO:31. This primer, primer 2, has the nucleic acid sequence 5' GATATGGAAC ATTTYACHCA ACAYAARGG 3', denoted SEQ ID NO:48, where R represents A or G. A PCR amplification product was generated using the combination of primer 2, SEQ ID NO:48, and the T7 standard  
10 primer, 5' GTAATACGAC TCACTATAGG GC 3', denoted SEQ ID NO:49. The resultant PCR product was subjected to DNA sequence analysis using standard techniques.

The PCR product was sequenced and found to contain 510 nucleotides, and is known as nDerf60<sub>510</sub>. The nucleotide sequence of the coding strand of nDerf60<sub>510</sub> is  
15 represented herein as SEQ ID NO:43, and its complement is denoted SEQ ID NO:45. Translation of SEQ ID NO:43 suggests that nDerf60<sub>510</sub> encodes a partial *D. farinae* 60-kD protein of about 170 amino acids, referred to herein as PDerf60<sub>170</sub>, with an amino acid sequence denoted SEQ ID NO:44, assuming an open reading frame in which the first codon spans from about nucleotide 1 through nucleotide 3 of SEQ ID NO:43, and  
20 the last codon spanning from about nucleotide 508 through about nucleotide 510 of SEQ ID NO:43. PDerf60<sub>170</sub> has an estimated molecular weight of 19.2 kD and an estimated pI of about 6.51.

Nucleic acid molecule nDerf60<sub>510</sub> was used as a probe to isolate a nucleic acid molecule that encodes a protein corresponding to a full-length, or larger partial *D.*  
25 *farinae* 60-kD protein. Using procedures described previously in Example 8, the whole *D. farinae* library was screened with the nucleic acid SEQ ID NO:43 radiolabeled with <sup>32</sup>P using standard techniques. Hybridization was done in 6X SSC, 5X Denhardt's

30 final wash of about 30 minutes in 0.2X SSC, 0.2% SDS.

PCR amplification was carried out on the primary phage plugs. Primer 1, denoted as SEQ ID NO:46, and T7 standard primer, denoted as SEQ ID NO:49, were used as the primers, and a PCR product was generated. Preliminary sequence analysis of this 1.6 kilobase PCR product showed that it represents a nucleic acid sequence that contains the complete sequence encoding the PDerf60 full-length protein.

Comparison of PDerf60<sub>170</sub>, the amino acid sequence of SEQ ID NO:44, with amino acid sequences reported in GenBank indicates that PDerf60<sub>170</sub> showed the most homology, i.e. about 39% identity, with a chitinase protein precursor from *Aphanodidium album*. (GenBank accession number P32470). Nucleic acid sequence SEQ ID NO:43 showed no significant homology to any of the sequences submitted to GenBank.

#### Example 11.

This example describes the isolation of nucleic acid molecules encoding *Dermatophagoides pteronyssius* 98 kD allergen protein.

Nucleic acid molecules with high homology to the *D. farinae* 98 kD allergen (map B) were isolated from a *D. pteronyssius* cDNA library by hybridization with a 32-P labeled cDNA encoding the *D. farinae* HMW-map composition.

A *D. pteronyssius* cDNA library was prepared as follows. Total RNA was extracted from approximately 2 grams of *D. pteronyssius* mites, using an acid-guanidium-phenol-chloroform method, described by Chomzynski et al., 1987, *Anal. Biochem* 162: pp 156-159. Poly A<sup>+</sup> selected RNA was separated from the total RNA preparation by oligo-dT cellulose chromatography using the mRNA Purification Kit (available from Pharmacia, Newark, NJ), according to the method recommended by the manufacturer. A whole *D. pteronyssius* cDNA library was constructed in lambda-Uni-ZAP<sup>TM</sup> XR vector (available from Stratagene, La Jolla, CA), using Stratagene's ZAP-cDNA Synthesis Kit protocol. Approximately 5 milligram (mg) of Poly A<sup>+</sup> RNA was used to produce the *D. pteronyssius* cDNA library.

Using a modification of the protocol described in the cDNA Synthesis Kit (available from Stratagene), the whole *D. pteronyssius* cDNA library was screened, using duplicate plaque lifts, with a 32P-labeled cDNA encoding the *D. farinae* 97 kD Map B allergen, i.e. SEQ ID NO:17. Hybridization was done in 6X SSC (for recipe see

Sambrook, et al., *ibid.*), 5X Denhardt's solution (for recipe see Sambrook, et al., *ibid.*), 0.5% sodium dodecyl sulfate (SDS) (available from Sigma), and 100 mg/ml of single stranded DNA (available from Sigma), at 55°C, for about 36 hours. The filters were washed 3 times, for about 30 minutes per wash, at 55°C, in 2X SSC, 0.2% SDS, followed by a final wash of about 30 minutes, at 55°C, in 0.2X SSC, 0.2% SDS. A plaque purified clone of the *D. pteronyssius* nucleic acid molecule encoding the *D. pteronyssius* 97 kD allergen (map B) was converted into a double stranded recombinant molecule using the ExAssist™ helper phage and SOLR™ *E. coli* according to the *in vivo* excision protocol described in the ZAP-cDNA Synthesis Kit (all available from Stratagene). The plasmid containing the *D. pteronyssius* clone was subjected to DNA sequence analysis using standard techniques. DNA sequence analysis, including the determination of molecular weight and isoelectric point (pI) was performed using the GCG™ program.

A clone was isolated that included an about 1621-nucleotide insert, which includes the full-length coding region, referred to herein as nDerp98<sub>1621</sub>, with a coding strand represented as SEQ ID NO:34 and a complementary strand represented as SEQ ID NO:36. The apparent start and stop codons span from nucleotide 14 through nucleotide 16, and from nucleotide 1541 through nucleotide 1543, respectively, of SEQ ID NO:34. A putative polyadenylation signal (5'AATAAA 3') is located in a region spanning from nucleotide 1584 to 1589 of SEQ ID NO:34.

Translation of SEQ ID NO:34 yields a protein of about 509 amino acids, denoted PDerp98<sub>509</sub>, the amino acid sequence of which is presented as SEQ ID NO:35. The nucleic acid molecule consisting of the coding region encoding PDerp98<sub>509</sub> is referred to herein as nDerp98<sub>1527</sub>, the nucleic acid sequence of which is represented as SEQ ID NO:37 (the coding strand), and SEQ ID NO:39 (the complementary strand). The amino acid sequence of PDerp98<sub>509</sub>, also represented herein as SEQ ID NO:38, has an estimated molecular weight of about 58.9 kD and an estimated pI of about 5.61

herein as PDerp98<sub>411</sub>, contains about 411 amino acids, and is represented herein as SEQ ID NO:41. The amino acid sequence of PDerp98<sub>411</sub> predicts the protein to have an

estimated molecular weight of about 56.8 kD, and an estimated pI of about 5.49, as well as two asparagine-linked glycosylation sites extending from about amino acid 115 to about amino acid 117, and extending from about amino acid 240 to amino acid 242, respectively. The nucleic acid molecule encoding PDerp98<sub>490</sub> is known as nDerp98<sub>1470</sub>,  
5 with a coding strand represented by SEQ ID NO:40 and a complementary strand represented by SEQ ID NO:42.

A BLAST search was performed as described previously. PDerp98<sub>509</sub>, SEQ ID NO:35, showed the highest homology at the amino acid level with the *Manduca sexta* chitinase (SwissProt accession number p36362), with about a 34% identity.

10 nDerp98<sub>1621</sub>, SEQ ID NO:34, showed the highest homology at the nucleic acid level to *Chelonus sp.* chitinase (accession number U10422), with about a 49% identity.

Comparison of cDNA regions corresponding to the coding regions for the *D. farinae* 98 kD allergen protein and the cDNA regions corresponding to the coding regions for the *D. pteronyssius* 98 kD allergen protein shows an identity of about 84%.

15 Example 14.

This example demonstrates the binding of the *D. farinae* HMW-map composition to human IgE in human sera isolated from humans known to be allergic to mite allergens.

A technique called RAST, or radio-allergo-absorbent test, was used because the  
20 amount of human IgE present in human sera is quite low. RAST was essentially performed as described in Aalberse, RC et al., (1981) *J. Allergy Clin Immunol.* 68: pp 356-364. To calculate the unit IU/ml, a standard curve was derived by performing RAST with several dilutions of a well-characterized chimeric human/mouse IgE monoclonal antibody against Derp2, (human IgE/monoclonal anti-Derp2, following the  
25 procedure of Schuurman, et al. (1997) *J Allergy Clin Immunol.* 99: pp 545-550).

Briefly, 50  $\mu$ g of the HMW-map composition, purified as described in Example 1, was coupled to 50 mg of CNBr-activated Sepharose 4B (available from Pharmacia, Piscataway, NJ), according to the manufacturer's protocols. Human sera were selected (17 different samples, total) on the basis of a positive RAST for whole mite *D. farinae*  
30 extracts, a positive RAST number is greater than 1 IU/ml). Two negative (less than 0.3 IU) control sera were also included.

To test each individual serum sample, 0.5 mg of the *D. farinae* HMW-map composition-coupled Sepharose was incubated with 50  $\mu$ l serum in a total volume of 300  $\mu$ l of PBS-T (Phosphate buffered saline with added 0.1% volume/volume Tween-20, available from Sigma). Incubation was overnight at 27°C, with shaking. After incubation, the coupled Sepharose was washed five times with PBS-T. Radiolabelled (<sup>125</sup>I-iodine) sheep anti-human IgE, made by standard radioiodination protocols, (diluted in PBS-T with 4.5% bovine serum and 0.5% sheep serum, v/v) in a total volume of 750  $\mu$ l, was added and incubated overnight at 27°C. After incubation, the coupled Sepharose was washed four times with PBS-T and counted in a gamma-counter to determine the amount of radiolabeled sheep anti-human IgE bound to the HMW-map composition-coupled Sepharose. The results are shown in Table 4.

Table 4 Binding of human IgE to HMW-map composition from *D. farinae*

Serum number	RAST, <i>D. farinae</i> whole extract, IU	RAST, HMW-map comps'n., IU
1445	>100	48
1456	>100	42
1458	21.1	0.5
1460	14.1	2.5
1463	37.6	0.1
1464	37.2	2.0
1465	14.5	0.7
1466	89.9	7.7
1468	>100	19.9
1471	31.9	0.8
1491	23.8	1.0
1496	25.3	3.6
1505	5.1	0.2
1523	1.0	<0.1
1529	1.2	0.7

Almost 75% of patients (11 of 15) who showed sensitivity to *D. farinae* whole mite extracts were sensitive to the HMW-map composition antigen, implying that the HMW-map composition antigen is a major antigen for *D. farinae* sensitive humans. Sensitivity to the HMW-map composition was defined as a RAST of greater than or  
5 equal to 0.5 IU.

#### Example 15.

This example demonstrates that the *D. farinae* HMW-map composition described in Example 1 includes a glycoprotein.

About (5.4  $\mu$ g) of a *D. farinae* HMW-map composition prepared in accordance  
10 with Example 1 was applied to SDS PAGE and electrophoresis was done according to standard techniques. The protein was blotted to a nitrocellulose membrane according to standard techniques, and glycoprotein was detected using the DIG™ Glycine Detection Kit (available from Boehringer Mannheim, Indianapolis, IN), using the manufacturer's protocol. The region corresponding to the HMW-map region showed a positive reaction  
15 with the kit, indicating that the HMW-map composition includes a glycoprotein.

#### Example 16.

This example shows that the *D. farinae* HMW-map composition retains its character as an allergen even when the amino acid residues are removed, both by chemical and enzymatic means. The results suggest that the main epitope(s) could be a  
20 carbohydrate epitope including a polysaccharide attached to an N-linked or O-linked glycosylation site on the HMW-map composition.

##### A. Protein elimination by chemical means ( $\beta$ -elimination of proteins)

Twelve  $\mu$ g (microgram) of HMW-map composition (purified as described in Example 1) was dissolved in 100  $\mu$ l (microliter) of distilled deionized water. To this  
25 mixture was added 5  $\mu$ l 10 M (molar) NaOH and 3.8 mg (milligram) NaBH<sub>4</sub> (available from Sigma) to give a final concentration of 0.5 M NaOH and 1 M NaBH<sub>4</sub>. This reaction mixture was heated at 50°C for 30 minutes, then cooled, and 100  $\mu$ l acetone was added. To this mixture, sufficient amount, i.e. approximately 150  $\mu$ l, of Dowex 50 (H+) (available from Pharmacia) was added to make the solution slightly acidic. The  
30 Dowex 50 adsorbed and removed the protein, leaving any sugar moieties in the supernatant. The mixture was centrifuged in a microcentrifuge and washed three times

with 100  $\mu$ l of water. The combined supernatants from the centrifugations were evaporated to dryness, then washed five times from a methanol:HCl solution (1000:1 v/v), evaporating to dryness after each wash, to remove salts. The mixture was dissolved in 100  $\mu$ l of water, and a portion (20  $\mu$ l) was analyzed by SDS-PAGE using standard techniques, and both Coomassie blue and Silver staining were used to determine the amount of protein in the chemically treated samples. No protein was detected by either Coomassie or Silver staining, indicating removal of protein. Any sugar moieties on the protein would be unaffected by these conditions.

The remainder of the residue from each sample was subjected to ELISA analysis as described in Example 4. Briefly, 100 ng of either the  $\beta$ -eliminated sample or of non- $\beta$ -eliminated sample of the HMW-map composition was coated onto the Immulon plates, and ELISAs were carried out as described in Example 4 with a *D. farinae* sensitive dog sera pool, a *D. farinae* sensitive cat sera pool, and various individual dog sera that are either *D. farinae* sensitive or not sensitive (as measured by ELISA). The results are shown in Table 5.

Table 5. Reactivity of dog and cat sera to HMW-map composition and to  $\beta$ -eliminated HMW-map composition (which is carbohydrate only)

Sera used	$\beta$ -eliminated HMW-map, OD (carbohydrate antigen)	untreated HMW-map comp's'n., OD X 10 <sup>-3</sup>
<i>D. farinae</i> dog pool	1233	1931
<i>D. farinae</i> cat pool	2837	3115
dog 1621A	15	0
dog 1621C	24	21
dog 1621S	59	420
dog 1626C	23	214
dog SPF-2	16	0

Results from Table 5 indicate that the  $\beta$ -eliminated HMW-map composition sample still retains the ability to bind IgE from dog and cat sera that is sensitive to *D. farinae* HMW-

#### B. Protein Elimination by enzymatic means.

14  $\mu$ g of HMW-map composition (purified as described in Example 1) was digested with 1  $\mu$ g Endoproteinase K, available from Sigma, to remove the protein moiety of the molecule. The digestion reaction took place at 56°C for 24 hours, after which the endoproteinase in the reaction was heat-denatured in boiling water for 10 minutes.

A portion of this reaction was analyzed by SDS-PAGE using standard techniques, and both Coomassie blue and Silver staining were used to detect the presence of protein in the enzymatically digested samples. No HMW-map composition was detected by either Coomassie or Silver staining, indicating elimination of the HMW-map composition. Any glycan that was attached via a glycosylation site on the protein would be unaffected by these conditions.

The remainder of the enzymatically digested reaction was tested by ELISA in the manner described in Example 4. Briefly, 100 ng of either the proteinase-K-digested sample or of a non-digested sample of the HMW-map composition was coated onto Immulon plates, and ELISAs were carried out as described in Example 4 with various individual dog sera that were either *D. farinae* sensitive or not sensitive (as measured by ELISA). The results are shown in Table 6.



Table 6. Reactivity of dog sera to HMW-map composition and to Endoproteinase-K digested HMW-map composition.

dog #	<i>D. farinae</i> sensitive? <sup>1</sup>	OD, wells coated with HMW-map comps'n.	OD, wells coated with Proteinase K digested HMW-map
1	yes	120	122
2	yes	1637	1561
3	yes	858	383
4	yes	914	509
5	yes	277	227
6	yes	2891	2636
7	no	10	11
8	yes	4056	3880
9	yes	1920	1626
10	yes	472	432
11	yes	328	213
12	yes	2913	2530
13	yes	1232	984
14	yes	3153	2355
15	no	6	46
16	yes	860	339
17	yes	2429	750
18	yes	1194	351
19	yes	2655	1443
20	yes	3285	1207
21	yes	2636	1240
22	yes	1097	848
23	yes	1621	1408
24	yes	2113	1592
25	yes	1169	408
26	yes	4200	4200
27	yes	4200	4200

28	no	0	4
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by ELISA in a separate experiment:

Results from Table 6 indicate that the proteinase-K digested HMW-map composition sample still retains the ability to bind IgE from dog and cat sera that is sensitive to *D. farinae* HMW-map composition, suggesting that the glycans attached to the protein constitute a major epitope on the HMW-map composition.

5 Example 17.

This example describes attempts to remove N-linked glycans from the HMW-map composition.

HMW-map composition (2  $\mu$ g), purified as in Example 1, was digested with N-glycosidase F (available from Boehringer-Mannheim), according to the manufacturer's  
10 directions. The digestion was analyzed by SDS-PAGE and stained according to standard protocols. 2  $\mu$ g Fetuin (available from Sigma) was used as a positive N-linked glycosylated protein control. Analysis of the SDS-PAGE showed that there were no apparent differences in the molecular weights of the intact and digested map B protein. The positive control, fetuin, did show a reduction of molecular weight after digestion  
15 with N-glycosidase F. This result indicates that there are no N-linked glycans on the HMW-map composition, or alternatively that there are only small sized N-glycans on the HMW-map composition.

While various embodiments of the present invention have been described in detail, it is apparent that modifications and adaptations of those embodiments will occur  
20 to those skilled in the art. It is to be expressly understood, however, that such modifications and adaptations are within the scope of the present invention, as set forth in the following claims.

What is claimed is:

1. An isolated nucleic acid molecule selected from the group consisting of:  
(a) a nucleic acid molecule comprising at least about 150 nucleotides, wherein said  
nucleic acid molecule comprising at least about 150 nucleotides hybridizes, in a solution  
comprising 1X SSC and 0% formamide, at a temperature of about 50°C, to a nucleic  
acid molecule comprising a nucleic acid sequence selected from the group consisting of  
SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:20, SEQ  
ID NO:22, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:39, SEQ ID  
NO:40, SEQ ID NO:42, SEQ ID NO:43, SEQ ID NO:45, and a nucleic acid sequence  
encoding a protein comprising the amino acid sequence of SEQ ID NO:33 and a  
complement thereof; and (b) a nucleic acid molecule comprising a fragment of any of  
said nucleic acid molecules of (a) wherein said fragment comprises at least about 15  
nucleotides.
2. An isolated protein encoded by a nucleic acid molecule selected from the  
group consisting of: (a) a nucleic acid molecule comprising at least about 150  
nucleotides, wherein said nucleic acid molecule comprising at least about 150  
nucleotides hybridizes, in a solution comprising 1X SSC and 0% formamide, at a  
temperature of about 50°C, to a nucleic acid molecule comprising a nucleic acid  
sequence selected from the group consisting of SEQ ID NO:16, SEQ ID NO:19, SEQ ID  
NO:22, SEQ ID NO:36, SEQ ID NO:39, SEQ ID NO:42, SEQ ID NO:45, and a  
complement of a nucleic acid sequence encoding a protein comprising the amino acid  
sequence SEQ ID NO:33; and (b) a nucleic acid molecule comprising a fragment of any  
of said nucleic acid molecules of (a), wherein said fragment comprises at least about 15  
nucleotides.
3. A therapeutic composition for treating an allergic response to a mite, said  
therapeutic composition comprising a desensitizing compound selected from the group  
consisting of: (a) an isolated mite allergenic protein, wherein said mite allergenic protein  
comprising an amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID  
NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ

ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:18, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:38, SEQ ID NO:41, and SEQ ID NO:44; (b) a mimotope of said mite allergenic protein; (c) a mutein of said mite allergenic protein; (d) an isolated nucleic acid molecule selected from the group consisting of: (i) a nucleic acid molecule comprising at least about 150 nucleotides, wherein said nucleic acid molecule comprising at least about 150 nucleotides hybridizes, in a solution comprising 1X SSC and 0% formamide, at a temperature of about 50°C, to a nucleic acid sequence selected from the group consisting of SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:43, SEQ ID NO:45 and a nucleic acid sequence encoding a protein comprising the amino acid sequence SEQ ID NO:33 and a complement thereof; and (ii) a nucleic acid molecule comprising a fragment of any of said nucleic acid molecules of (i), wherein said fragment comprises at least about 15 nucleotides; (e) an antibody to said mite allergenic protein; and (f) an inhibitor of binding of said mite allergenic protein to IgE.

4. An assay kit for testing if an animal is susceptible to or has an allergic response to a mite, said kit comprising:

(a) an isolated protein encoded by a nucleic acid molecule that hybridizes under stringent hybridization conditions with the complement of a nucleic acid molecule that encodes an amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:18, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:38, SEQ ID NO:41, and SEQ ID NO:44; and

(b) a means for determining if said animal is susceptible to or has said allergic response, wherein said means comprises use of said protein to identify animals susceptible to or having allergic responses to mites.

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5. A method to identify an animal susceptible to or having an allergic response to a mite, said method comprising:

- (a) contacting an isolated protein that is encoded by a nucleic acid molecule that hybridizes under stringent hybridization conditions with the complement of a nucleic acid molecule that encodes an amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:18, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:38, SEQ ID NO:41, and SEQ ID NO:44 with antibodies of an animal; and
- (b) determining immunocomplex formation between said protein and said antibodies, wherein formation of said immunocomplex indicates that said animal is susceptible to or has said allergic response.

6. A method to desensitize a host animal to an allergic response to a mite, said method comprising administering to said animal a therapeutic composition comprising a desensitizing compound selected from the group consisting of: (a) an isolated mite allergenic protein, wherein said mite allergenic protein is encoded by a nucleic acid molecule that hybridizes under stringent hybridization conditions with the complement of a nucleic acid molecule that encodes an amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:18, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:38, SEQ ID NO:41, and SEQ ID NO:44; (b) a mimotope of said mite allergenic protein; (c) a mutein of said mite allergenic protein; (d) an isolated nucleic acid

about 150 nucleotides hybridizes, in a solution comprising 1X SSC and 0.1% formamide, at a temperature of about 50°C, to a nucleic acid sequence selected from the group

consisting of SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:43, SEQ ID NO:45 and a nucleic acid sequence encoding a protein comprising the amino acid sequence SEQ ID NO:33 and a complement thereof; and (ii) a nucleic acid molecule comprising a fragment of any of said nucleic acid molecules of (i), wherein said fragment comprises at least about 15 nucleotides; (e) an antibody to said mite allergic protein; and (f) an inhibitor of binding of said mite allergic protein to IgE.

7. A method to produce a mite allergenic protein, said method comprising culturing a cell transformed with a nucleic acid molecule selected from the group consisting of: a nucleic acid molecule comprising at least about 150 nucleotides, wherein said nucleic acid molecule comprising at least about 150 nucleotides hybridizes, in a solution comprising 1X SSC and 0% formamide, at a temperature of about 50°C, to a nucleic acid sequence selected from the group consisting of SEQ ID NO:16, SEQ ID NO:19, SEQ ID NO:22, SEQ ID NO:36, SEQ ID NO:39, SEQ ID NO:42, SEQ ID NO:45 and a complement of a nucleic acid sequence encoding a protein comprising the amino acid sequence SEQ ID NO:33; and a nucleic acid molecule comprising a fragment of any of said nucleic acid molecules, wherein said fragment comprises at least about 15 nucleotides.

8. A reagent comprising a non-proteinaceous epitope having at least one identifying characteristic selected from the group consisting of:

- (a) said epitope is resistant to  $\beta$ -elimination of peptides;
- (b) said epitope is resistant to Proteinase-K digestion; and
- (c) said epitope is reactive to a test designed to detect glycosylated proteins,

wherein said epitope binds to an IgE selected from the group consisting of canine IgE from dogs allergic to mites and feline IgE from cats allergic to mites.

9. The invention of Claim 1, 2, 3, 4, 5, 6, or 7, wherein said nucleic acid molecule comprises a nucleic acid sequence that encodes a *Der* HMW-map protein.

10. The invention of Claim 1, 2, 3, 4, 5, 6, or 7, wherein said nucleic acid molecule is selected from the group consisting of nDerf98<sub>1752</sub>, nDerf98<sub>1665</sub>, nDerf98<sub>1608</sub>, nDerp98<sub>1621</sub>, nDerp98<sub>1527</sub>, nDerp98<sub>1470</sub>, and nDerf60<sub>510</sub>.

11 The invention of Claim 1, 2, 3, 4, 5, 6, or 7, wherein said nucleic acid molecule is selected from the group consisting of: (a) a nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:43, SEQ ID NO:45; and (b) a nucleic acid molecule comprising an allelic variant of a nucleic acid molecule of (a).

12 The invention of Claim 1, 2, 3, 4, 5, 6, or 7, wherein said nucleic acid molecule is selected from the group consisting of: (a) a nucleic acid molecule comprising a nucleic acid sequence that encodes a protein having an amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:18, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:38, SEQ ID NO:41, and SEQ ID NO:44; and (b) a nucleic acid molecule comprising an allelic variant of a nucleic acid molecule encoding a protein having an amino acid sequence of (a).

13 A recombinant molecule comprising a nucleic acid molecule as set forth in Claim 1, 2, 3, 4, 5, 6, or 7 operatively linked to a transcription control sequence.

14 A recombinant virus comprising a nucleic acid molecule as set forth in Claim 1, 2, 3, 4, 5, 6, or 7.

15 A recombinant cell comprising a nucleic acid molecule as set forth in Claim 1, 2, 3, 4, 5, 6, or 7.

16 The invention of Claim 2, 3, 4, 5, 6, or 7, wherein said protein, when administered to an animal, elicits an immune response against a *Der* HMW-map protein.

17 The invention of Claim 2, 3, 4, 5, 6, or 7, wherein said protein is selected

NO:17, SEQ ID NO:21, SEQ ID NO:34, SEQ ID NO:37, SEQ ID NO:40, SEQ ID NO:43, and the coding strand of a nucleic acid sequence encoding a protein comprising

the amino acid sequence SEQ ID NO:33; and (b) a protein encoded by a nucleic acid molecule comprising an allelic variant of a nucleic acid molecule comprising any of said nucleic acid molecules of (a).

18. The invention of Claim 2, 3, 4, 5, 6, or 7, wherein said protein is selected  
5 from the group consisting of: (a) a protein comprising an amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:18, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:29, SEQ ID  
10 NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:38, SEQ ID NO:41, and SEQ ID NO:44; and (b) a protein encoded by an allelic variant of a nucleic acid molecule encoding a protein comprising any of said amino acid sequences of (a).

19. An isolated antibody that selectively binds to a protein as set forth in  
15 Claim 2, 3, 4, 5, 6, or 7.

20. The protein of Claim 2, 3, 4, 5, 6, or 7, wherein said protein selectively binds to IgE.

21. The protein of Claim 2, 3, 4, 5, 6, or 7, wherein said protein comprises an epitope having at least one identifying characteristic selected from the group consisting  
20 of:

- (a) said epitope is resistant to  $\beta$ -elimination of peptides;
- (b) said epitope is resistant to Proteinase-K digestion; and
- (c) said epitope is reactive to a test designed to detect glycosylated proteins,

25 wherein said epitope binds to an IgE selected from the group consisting of canine IgE from dogs allergic to mites and feline IgE from cats allergic to mites.

22. The invention of Claim 3 or 6, wherein said desensitizing compound is administered to an animal as a naked nucleic acid molecule.

23. A therapeutic composition for treating an allergic response to a mite, said  
30 therapeutic composition comprising a desensitizing compound comprising the reagent of Claim 8.



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24. An assay kit for testing if an animal is susceptible to or has an allergic response to a mite, said kit comprising the reagent of Claim 8 and a means for determining if said animal is susceptible to or has said allergic response, wherein said means comprises use of said reagent to identify animals susceptible to or having allergic responses to mites.

25. A method to identify an animal susceptible to or having an allergic response to a mite, said method comprising:

(a) contacting the reagent of Claim 8 with antibodies of an animal; and

(b) determining immunocomplex formation between said reagent and said antibodies, wherein formation of said immunocomplex indicates that said animal is susceptible to or has said allergic response.

26. A method to desensitize a host animal to an allergic response to a mite, said method comprising administering to said animal a therapeutic composition comprising a desensitizing compound comprising the reagent of Claim 8.

27. An isolated antibody that selectively binds to an epitope as set forth in Claim 8.

28. The invention of Claim 3, 6, 23, or 26, wherein said composition further comprises a component selected from the group consisting of an excipient, an adjuvant, and a carrier.

29. The invention of Claim 4, 5, 24, or 25, wherein said means of Claim 4 or 24 or said step of contacting of Claim 5 or 25 is performed *in vitro* or *in vivo*.

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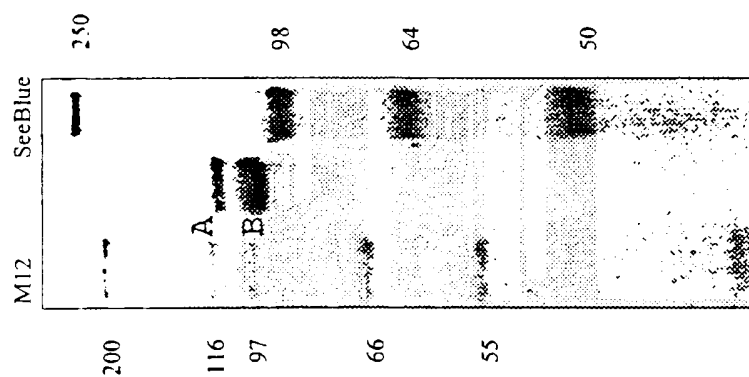


Fig. 1

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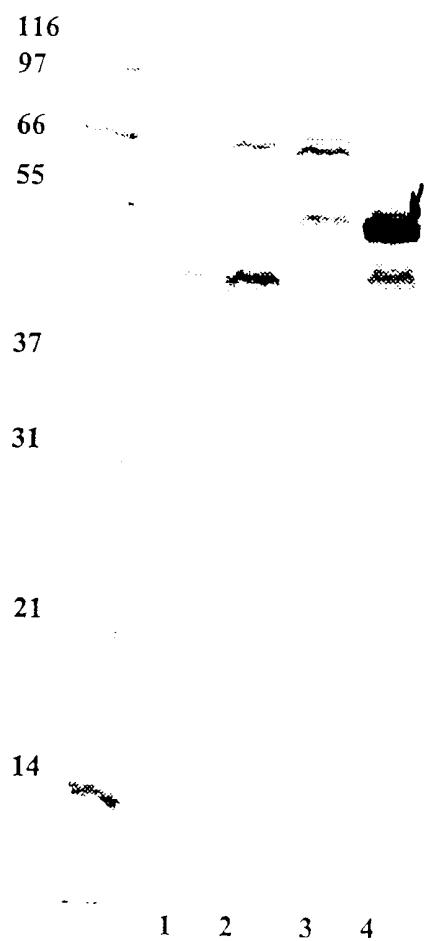


Fig. 2

## SEQUENCE LISTING

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Hunter, Shirley Wu  
Weber, Eric R.

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tttatacaac ggagcattgt gacgtaaaa gttttcccat ccaccgtggt aatcatatgt 1080  
catgacattc atccaatcga acaatttgtt caattctttg atatcataag ctgggtcgat 1140  
tttctcttta cctgggtgata ctgcagcagt caacaagtag ccatgagggt caaaagcgtc 1200  
tttaagttct ctaaccaaag ccaaatagtt ttgtttatcg attttcgggt taccoaatcg 1260  
agatccagga tactcccaat ccaaattctag accgtcgaac ttgtattctt gcaaaaagtc 1320  
caaaactgat tgtatgaatt gttgaogata tgttggattt gcagccatat cggaatattt 1380  
ttccgagcct tcataccaac caccaagtga aatcatgggt gttaattctg gattcttcaa 1440  
tcgcaagttg ttgaaacgtt cataaccacg tttttcccat gagttatggt tatcatcttg 1500  
gtaaggatcg aaaacttgaa ttgtgtattt gtattcatca attttagcga aaccatacat 1560  
taaagtgtga cacttgaatg gatcaatatt ttcatagtg tatggatcaa ctttatgata 1620  
tacggaccat gttccaacat aacaaacaat tctcatcgga tttttcgaat aatcattatg 1680  
atctcgtttg atggatgcat tcataaggcc aatgcaggcc ataatactaa gtattgcata 1740  
tatggttttc at 1752

&lt;210&gt; 17

&lt;211&gt; 1665

&lt;212&gt; DNA

&lt;213&gt; Dermatophagoides farinae

&lt;220&gt;

&lt;221&gt; CDS

&lt;222&gt; 1..(1665)

&lt;400&gt; 17

atg aag aac ata tat gca ata ctt agt att atg gcc tgc att ggc ctt 48

Met Lys Thr Ile Tyr Ala Ile Leu Ser Ile Met Ala Cys Ile Gly Leu

1

5

10

15

ctg aat cca tcc atc aaa cga gat cat aat gat tat tcg aaa aat ccg 96

Met Asn Ala Ser Ile Lys Arg Asp His Asn Asp Tyr Ser Lys Asn Pro

20

25

30

atg agc att ctt tgt tat gtt gga aca tgg tcc gta tat cat aaa gtt 144

Met Arg Ile Val Cys Tyr Val Gly Thr Trp Ser Val Tyr His Lys Val

35

40

45

gat cca tac act atc gaa gat att gat cca ttc aag tgt aca cat tta 192

Asp Pro Tyr Thr Ile Glu Asp Ile Asp Pro Phe Lys Cys Thr His Leu

50

55

60

atg tat ggt ttc gct aaa att gat gaa tac aaa tac aca att caa gtt 240

Met Tyr Gly Phe Ala Lys Ile Asp Glu Tyr Lys Tyr Thr Ile Gln Val

65

70

75

80

ttc gat cct tac caa gat gat aac cat aac tca tgg gaa aaa cgt ggt 288

Phe Asp Pro Tyr Gln Asp Asp Asn His Asn Ser Trp Glu Lys Arg Gly

85

90

95

tat gaa cgt ttc aac aac ttg cga ttg aag aat cca gaa tta acc acc 336

Tyr Glu Arg Phe Asn Asn Leu Arg Leu Lys Asn Pro Glu Leu Thr Thr

100

105

110

atg att tca ctt ggt ggt tgg tat gaa ggc tcg gaa aaa tat tcc gat 384

Met Ile Ser Leu Gly Gly Trp Tyr Glu Gly Ser Glu Lys Tyr Ser Asp

115

120

125

atg gct gca aat cca aca tat cgt caa caa ttc ata caa tca gtt ttg 432

(3)



tat tac aat gct cca tat ggt tac aat gat aaa atc tgg gtc ggt tac	1056
Tyr Tyr Asn Ala Pro Tyr Gly Tyr Asn Asp Lys Ile Trp Val Gly Tyr	
340 345 350	
gat gat ctg gcc agt ata tca tgc aag ttg gct ttc ctg aaa gaa tta	1104
Asp Asp Leu Ala Ser Ile Ser Cys Lys Leu Ala Phe Leu Lys Glu Leu	
355 360 365	
ggc gtt tct ggt gtc atg gtt tgg tca ttg gaa aat gat gat ttc aaa	1152
Gly Val Ser Gly Val Met Val Trp Ser Leu Glu Asn Asp Asp Phe Lys	
370 375 380	
ggt cac tgc gga ccg aaa aat cca ttg ttg aac aaa gtt cat aat atg	1200
Gly His Cys Gly Pro Lys Asn Pro Leu Leu Asn Lys Val His Asn Met	
385 390 395 400	
att aat ggc gat gaa aag aac tct ttc gaa tgc att ttg ggt cca agt	1248
Ile Asn Gly Asp Glu Lys Asn Ser Phe Glu Cys Ile Leu Gly Pro Ser	
405 410 415	
aca acg aca cca act cca acg acg aca ccc aca acc ccg act aca acg	1296
Thr Thr Thr Pro Thr Pro Thr Thr Thr Pro Thr Thr Pro Thr Thr Thr	
420 425 430	
cca aca act cct tct ccc acc acc ccg aca aca acc cct tct ccc acc	1344
Pro Thr Thr Pro Ser Pro Thr Thr Pro Thr Thr Thr Pro Ser Pro Thr	
435 440 445	
acc ccg aca aca acc cct tct ccc acc aca ccg aca aca act cct tct	1392
Thr Pro Thr Thr Thr Pro Ser Pro Thr Thr Pro Thr Thr Thr Pro Ser	
450 455 460	
ccc acc aca cca aca cca aca aca cca aca cca gcc cct aca aca tgg	1440
Pro Thr Thr Pro Thr Pro Thr Thr Pro Thr Pro Ala Pro Thr Thr Ser	
465 470 475 480	
aca cct tgg cca acc acg acc gaa cac aca agc gaa aca cca aaa tat	1488
Thr Pro Ser Pro Thr Thr Thr Glu His Thr Ser Glu Thr Pro Lys Tyr	
485 490 495	
aca acc tat gtc gat gga cat ctt atc aaa tgt tac aag gaa ggt gat	1536
Thr Thr Tyr Val Asp Gly His Leu Ile Lys Cys Tyr Lys Glu Gly Asp	
500 505 510	
atc cca cat cca acc aat ata ccc aaa tat ttc gtc tgt gaa ttt ctt	1584

aat ggt ggc tgg tgg gtt cat att atg ccc tgt cca ccg ggc act att 1632  
 Asn Gly Gly Trp Trp Val His Ile Met Pro Cys Pro Pro Gly Thr Ile  
 530 535 540

tgg tgt caa gaa aaa ttg act tgt ata ggc gaa 1665  
 Trp Cys Gln Glu Lys Leu Thr Cys Ile Gly Glu  
 545 550 555

110-18  
 111-559  
 112-FAST  
 113-*Teratophagoides farinae*

400-18  
 Met Lys Thr Ile Tyr Ala Ile Leu Ser Ile Met Ala Cys Ile Gly Leu  
 1 5 10 15

Met Asn Ala Ser Ile Lys Arg Asp His Asn Asp Tyr Ser Lys Asn Pro  
 20 25 30

Met Arg Ile Val Cys Tyr Val Gly Thr Trp Ser Val Tyr His Lys Val  
 35 40 45

Asp Pro Tyr Thr Ile Glu Asp Ile Asp Pro Phe Lys Cys Thr His Leu  
 50 55 60

Met Tyr Gly Phe Ala Lys Ile Asp Glu Tyr Lys Tyr Thr Ile Gln Val  
 65 70 75 80

Phe Asp Pro Tyr Gln Asp Asp Asn His Asn Ser Trp Glu Lys Arg Gly  
 85 90 95

Tyr Glu Arg Phe Asn Asn Leu Arg Leu Lys Asn Pro Glu Leu Thr Thr  
 100 105 110

Met Ile Ser Leu Gly Gly Trp Tyr Glu Gly Ser Glu Lys Tyr Ser Asp  
 115 120 125

Met Ala Ala Asn Pro Thr Tyr Arg Gln Gln Phe Ile Gln Ser Val Leu  
 130 135 140

Asp Phe Leu Gln Glu Tyr Lys Phe Asp Gly Leu Asp Leu Asp Trp Glu  
 145 150 155 160

Tyr Pro Gly Ser Arg Leu Gly Asn Pro Lys Ile Asp Lys Gln Asn Tyr  
 165 170 175

Leu Ala Leu Val Arg Glu Leu Lys Asp Ala Phe Glu Pro His Gly Tyr  
 180 185 190

Leu Leu Thr Ala Ala Val Ser Pro Gly Lys Asp Lys Ile Asp Arg Ala  
 195 200 205

Tyr Asp Ile Lys Glu Leu Asn Lys Leu Phe Asp Trp Met Asn Val Met  
 210 215 220

Thr Tyr Asp Tyr His Gly Gly Trp Glu Asn Phe Tyr Gly His Asn Ala  
 225 230 235 240

Pro Leu Tyr Lys Arg Pro Asp Glu Thr Asp Glu Leu His Thr Tyr Phe  
 245 250 255

Asn Val Asn Tyr Thr Met His Tyr Tyr Leu Asn Asn Gly Ala Thr Arg  
 260 265 270

Asp Lys Leu Val Met Gly Val Pro Phe Tyr Gly Arg Ala Trp Ser Ile  
 275 280 285

Glu Asp Arg Ser Lys Leu Lys Leu Gly Asp Pro Ala Lys Gly Met Ser  
 290 295 300

Pro Pro Gly Phe Ile Ser Gly Glu Glu Gly Val Leu Ser Tyr Ile Glu  
 305 310 315 320

Leu Cys Gln Leu Phe Gln Lys Glu Glu Trp His Ile Gln Tyr Asp Glu  
 325 330 335

Tyr Tyr Asn Ala Pro Tyr Gly Tyr Asn Asp Lys Ile Trp Val Gly Tyr  
 340 345 350

Asp Asp Leu Ala Ser Ile Ser Cys Lys Leu Ala Phe Leu Lys Glu Leu  
 355 360 365

Gly Val Ser Gly Val Met Val Trp Ser Leu Glu Asn Asp Asp Phe Lys  
 370 375 380

Gly His Cys Gly Pro Lys Asn Pro Leu Leu Asn Lys Val His Asn Met  
 385 390 395 400

Ile Asn Gly Asp Glu Lys Asn Ser Phe Glu Cys Ile Leu Gly Pro Ser  
 405 410 415

Pro Thr Thr Pro Ser Pro Thr Thr Pro Thr Thr Thr Pro Ser Pro Thr  
 435 440 445  
 Thr Pro Thr Thr Thr Pro Ser Pro Thr Thr Pro Thr Thr Thr Pro Ser  
 450 455 460  
 Pro Thr Thr Pro Thr Pro Thr Thr Pro Thr Pro Ala Pro Thr Thr Ser  
 465 470 475 480  
 Thr Pro Ser Pro Thr Thr Thr Glu His Thr Ser Glu Thr Pro Lys Tyr  
 485 490 495  
 Thr Thr Tyr Val Asp Gly His Leu Ile Lys Cys Tyr Lys Glu Gly Asp  
 500 505 510  
 Ile Pro His Pro Thr Asn Ile His Lys Tyr Leu Val Cys Glu Phe Val  
 515 520 525  
 Asn Gly Gly Trp Trp Val His Ile Met Pro Cys Pro Pro Gly Thr Ile  
 530 535 540  
 Trp Cys Gln Glu Lys Leu Thr Cys Ile Gly Glu  
 545 550 555

&lt;210&gt; 19

&lt;211&gt; 1665

&lt;212&gt; DNA

&lt;213&gt; Dermatophagoides farinae

&lt;400&gt; 19

ttgcgctata caagtcatt tttcttgaca ccaaatagtg cccggtggac agggcataat 60  
 atgaaccac cagccacat taacaaattc acagacaaaa tatttgtgta tatttggttg 120  
 atgtgggata tcaccttcct tgtaacattt gataagatgt ccatcgacat aggttgtata 180  
 ttttggtgtt tcgcttgtgt gttcggtcgt ggttgccgaa ggtgtcgatg ttgtaggggc 240  
 tgggtgttggg gttgttggtg ttggtgtggg gggagaagga gttgttgtcg gtgtggtggg 300  
 agaaggggtt gttgtcgggg tgggtggaga aggggttgtt gtcgggggtg tgggagaagg 360  
 agttgttggc gttgtagtcg ggggtgtggg tgcgtcgtt ggagttggtg tcgttgtact 420  
 tggacccaaa atgcattoga aagagttcct ttcacggcca ttaatcatat tatgaacttt 480  
 gttcaacaat ggatttttcg gtccgcagtg accttgaaa tcatcatttt ccaatgacca 540

aaccatgaca ccagaaaagc ctaattcttt caggaaaagcc aacttgcacg atatactggc 600  
cagatcatcg taaccgaccc agattttatc attgtaacca tatggagcat tgtaatatcc 660  
atcgtattgg atatgacatt cttctttttg aaacaattga cacaattcta tatatgagag 720  
gacaccttct tcaccagaaa tgaaacctgg gggcgacatg cttttggctg gatctccaag 780  
tttgagtctg ctccgatctt caatgctcca agcacggcca tagaatggaa caccattac 840  
caatttgtct ctgggtggac cattgttcaa ataatagtgc atgggtgtagt tgacattgaa 900  
gtaagtgtgc aactcatcag ttccatctgg tcgtttatac aacggagcat tgtgaccgta 960  
aaagttttcc catccacccg ggtaactata tgcacgaca ttccatccat cgaacaattt 1020  
gttcaattct ttgatatcat aagctcggc gattttgtct ttacctggtg atactgcagc 1080  
agtcaacaag tagccatgag gtccaaaagc gtctttaagt tctctaacca aagccaaata 1140  
gttttgttta tcgattttcg ggttacccaa tcgagatcca ggatactccc aatccaaatc 1200  
tagaccgtcg aacttgtatt cttgcaaaaa gtccaaaact gattgtatga attgttgacg 1260  
atatgttgga ttgcagcca tatcggaata tttttccgag ctttcatacc aaccaccaag 1320  
tgaaatcatg gtgggttaatt ctggattctt caatcgcaag ttgttgaaac gttcataacc 1380  
acgtttttcc catgagttat ggttatcacc ttggtaagga tcgaaaactt gaattgtgta 1440  
tttgtattca tcaattttag cgaaaccata cattaaatgt gtacacttga atggatcaat 1500  
atcttcgata gtgtatggat caactttatg atatacggac catgttccaa cataacaaac 1560  
aattctcacc ggatttttcg actaaacatt atgatctcgt ttgatggatg cattcataag 1620  
gcacatgcag gcacataacc taagtattcc atatatggtt ttcac 1665

<210> 20

<211> 1608

<212> DNA

<213> *Dermatophagoides farinae*

&lt;400&gt; 20

tcc atc aaa cga gat cat aat gat tat tcg aaa aat ccg atg aga att	48
Ser Ile Lys Arg Asp His Asn Asp Tyr Ser Lys Asn Pro Met Arg Ile	
1 5 10 15	
ggt tgt tat gtt gga aca tgg tcc gta tat cat aaa gtt gat cca tac	96
Val Cys Tyr Val Gly Thr Trp Ser Val Tyr His Lys Val Asp Pro Tyr	
20 25 30	
act atc gaa gat att gat cca ttc aag tgt aca cat tta atg tat ggt	144
Thr Ile Glu Asp Ile Asp Pro Phe Lys Cys Thr His Leu Met Tyr Gly	
35 40 45	
ttc gct aaa att gat gaa tac aaa tac aca att caa gtt ttc gat cct	192
Phe Ala Lys Ile Asp Glu Tyr Lys Tyr Thr Ile Gln Val Phe Asp Pro	
50 55 60	
tac caa gat gat aac cat aac tca tgg gaa aaa cgt ggt tat gaa cgt	240
Tyr Gln Asp Asp Asn His Asn Ser Trp Glu Lys Arg Gly Tyr Glu Arg	
65 70 75 80	
ttc aac aac ttg cga ttg aag aat cca gaa tta acc acc atg att tca	288
Phe Asn Asn Leu Arg Leu Lys Asn Pro Glu Leu Thr Thr Met Ile Ser	
85 90 95	
ctt ggt ggt tgg tat gaa ggc tcg gaa aaa tat tcc gat atg gct gca	336
Leu Gly Gly Trp Tyr Glu Gly Ser Glu Lys Tyr Ser Asp Met Ala Ala	
100 105 110	
aat cca aca tat cgt caa caa ttc ata caa tca gtt ttg gac ttt ttg	384
Asn Pro Thr Tyr Arg Gln Gln Phe Ile Gln Ser Val Leu Asp Phe Leu	
115 120 125	
caa gaa tac aag ttc gac ggt cta gat ttg gat tgg gag tat cct gga	432
Gln Glu Tyr Lys Phe Asp Gly Leu Asp Leu Asp Trp Glu Tyr Pro Gly	
130 135 140	
tct cga ttg ggt aac ccg aaa atc gat aaa caa aac tat ttg gct ttg	480
Ser Arg Leu Gly Asn Pro Lys Ile Asp Lys Gln Asn Tyr Leu Ala Leu	
145 150 155 160	
ggt aga gaa ctt aaa gac gct ttt gaa cct cat ggc tac ttg ttg act	528
Val Arg Glu Leu Lys Asp Ala Phe Glu Pro His Gly Tyr Leu Leu Thr	
165 170 175	
gct gca gta tca cca ggt aaa gac aaa atc gac cga gct tat gat atc	576
Ala Ala Val Ser Pro Gly Lys Asp Lys Ile Asp Arg Ala Tyr Asp Ile	

180	185	190	
aaa gaa ttg aac aaa ttg ttc gat tgg atg aat gtc atg aca tat gat			624
Lys Glu Leu Asn Lys Leu Phe Asp Trp Met Asn Val Met Thr Tyr Asp			
195	200	205	
tac cac ggt gga tgg gaa aac ttt tac ggt cac aat gct ccg ttg tat			672
Tyr His Gly Gly Trp Glu Asn Phe Tyr Gly His Asn Ala Pro Leu Tyr			
210	215	220	
aaa cga cca gat gaa act gat gag ttg cac act tac ttc aat gtc aac			720
Lys Arg Pro Asp Glu Thr Asp Glu Leu His Thr Tyr Phe Asn Val Asn			
225	230	235	240
tac acc atg cac tat tat ttg aac aat ggt gcc acc aga gac aaa ttg			768
Tyr Thr Met His Tyr Tyr Leu Asn Asn Gly Ala Thr Arg Asp Lys Leu			
245	250	255	
gta atg ggt gtt cca ttc tat ggc cgt gct tgg agc att gaa gat cga			816
Val Met Gly Val Pro Phe Tyr Gly Arg Ala Trp Ser Ile Glu Asp Arg			
260	265	270	
agc aaa ctc aaa ctt gga gat cca gcc aaa ggc atg tcg ccc cca ggt			864
Ser Lys Leu Lys Leu Gly Asp Pro Ala Lys Gly Met Ser Pro Pro Gly			
275	280	285	
ttc att tct ggt gaa gaa ggt gtc ctc tca tat ata gaa ttg tgt caa			912
Phe Ile Ser Gly Glu Glu Gly Val Leu Ser Tyr Ile Glu Leu Cys Gln			
290	295	300	
ttg ttt caa aaa gaa gaa tgg cat atc caa tac gat gaa tat tac aat			960
Leu Phe Gln Lys Glu Glu Trp His Ile Gln Tyr Asp Glu Tyr Tyr Asn			
305	310	315	320
gct cca tat ggt tac aat gat aaa atc tgg gtc ggt tac gat gat ctg			1008
Ala Pro Tyr Gly Tyr Asn Asp Lys Ile Trp Val Gly Tyr Asp Asp Leu			
325	330	335	
gcc agt ata tca tgc aaq ttg ggt ttc ctg aaa gaa tta ggc gtt tct			1056
Ala Ser Ile Ser Cys Lys Leu Ala Phe Leu Lys Glu Leu Gly Val Ser			
340	345	350	
ggg gtc atg gtt tgg tca ttg gaa aat gat gat ttc aaa ggt cac tgc			1104
Gly Val Met Val Trp Ser Leu Glu Asn Asp Asp Phe Lys Gly His Cys			
355	360	365	

370	375	380	
gat gaa aag aac tct ttc gaa tgc att ttg ggt cca agt aca acg aca	1200		
Asp Glu Lys Asn Ser Phe Glu Cys Ile Leu Gly Pro Ser Thr Thr Thr			
385	390	395	400
cca act cca acg acg aca ccc aca acc ccg act aca acg cca aca act	1248		
Pro Thr Pro Thr Thr Thr Pro Thr Thr Thr Thr Thr Pro Thr Thr			
405	410	415	
cct tct ccc acc acc ccg aca aca acc cct tct ccc acc acc ccg aca	1296		
Pro Ser Pro Thr Thr Thr Thr Thr Pro Ser Pro Thr Thr Pro Thr			
420	425	430	
aca acc cct tct ccc acc aca ccg aca aca act cct tct ccc acc aca	1344		
Thr Thr Pro Ser Pro Thr Thr Pro Thr Thr Thr Pro Ser Pro Thr Thr			
435	440	445	
cca aca cca aca aca cca aca cca gcc cct aca aca tcg aca cct tcg	1392		
Pro Thr Pro Thr Thr Pro Thr Pro Ala Pro Thr Thr Ser Thr Pro Ser			
450	455	460	
cca acc acg acc gaa cac aca agc gaa aca cca aaa tat aca acc tat	1440		
Pro Thr Thr Thr Glu His Thr Ser Glu Thr Pro Lys Tyr Thr Thr Tyr			
465	470	475	480
gtc gat gga cat ctt atc aaa tgt tac aag gaa ggt gat atc cca cat	1488		
Val Asp Gly His Leu Ile Lys Cys Tyr Lys Glu Gly Asp Ile Pro His			
485	490	495	
cca acc aat ata cac aaa tat ttg gtc tgt gaa ttt gtt aat ggt ggc	1536		
Pro Thr Asn Ile His Lys Tyr Leu Val Cys Glu Phe Val Asn Gly Gly			
500	505	510	
tgg tgg gtt cat att atg ccc tgt cca ccg ggc act att tgg tgt caa	1584		
Trp Trp Val His Ile Met Pro Cys Pro Pro Gly Thr Ile Trp Cys Gln			
515	520	525	
gaa aaa ttg act tgt ata ggc gaa	1608		
Glu Lys Leu Thr Cys Ile Gly Glu			
530	535		

&lt;210&gt; 21

&lt;211&gt; 536

&lt;212&gt; PRT

&lt;213&gt; Dermatophagoides farinae



&lt;400&gt; 21

Ser Ile Leu Arg Asp His Asn Asp Tyr Ser Lys Asn Pro Met Arg Ile  
1 5 10 15

Val Cys Thr Val Thr Trp Ser Val Tyr His Lys Val Asp Pro Tyr  
20 25 30

Thr Ile Val Asn Thr Asp Pro Phe Lys Cys Thr His Leu Met Tyr Gly  
35 40 45

Phe Ala Leu Val Asp Glu Tyr Lys Tyr Thr Ile Gln Val Phe Asp Pro  
50 55 60

Tyr Gln Asn Asp Thr Asn Ser Trp Glu Lys Arg Gly Tyr Glu Arg  
65 70 75 80

Phe Asn Asn Val Thr Leu Asn Pro Glu Leu Thr Thr Met Ile Ser  
85 90 95

Leu Gly Gly Thr Thr Gly Ser Glu Lys Tyr Ser Asp Met Ala Ala  
100 105 110

Asn Pro Thr Thr Val Gln Gln Phe Ile Gln Ser Val Leu Asp Phe Leu  
115 120 125

Gln Glu Tyr Lys Thr Asp Gly Leu Asp Leu Asp Trp Glu Tyr Pro Gly  
130 135 140

Ser Arg Leu Gly Asn Pro Lys Ile Asp Lys Gln Asn Tyr Leu Ala Leu  
145 150 155 160

Val Arg Glu Leu Asp Asp Ala Phe Glu Pro His Gly Tyr Leu Leu Thr  
165 170 175

Ala Ala Val Ser Thr Gly Lys Asp Lys Ile Asp Arg Ala Tyr Asp Ile  
180 185 190

Lys Glu Leu Asn Lys Leu Phe Asp Trp Met Asn Val Met Thr Tyr Asp  
195 200 205

Tyr His Gly Gly Trp Glu Asn Phe Tyr Gly His Asn Ala Pro Leu Tyr  
210 215 220

Lys Arg Pro Asp Glu Thr Asp Glu Leu His Thr Tyr Phe Asn Val Asn  
225 230 235 240

Val Met Gly Val Pro Phe Tyr Gly Arg Ala Trp Ser Ile Glu Asp Arg  
 260 265 270  
 Ser Lys Leu Lys Leu Gly Asp Pro Ala Lys Gly Met Ser Pro Pro Gly  
 275 280 285  
 Phe Ile Ser Gly Glu Glu Gly Val Leu Ser Tyr Ile Glu Leu Cys Gln  
 290 295 300  
 Leu Phe Gln Lys Glu Glu Trp His Ile Gln Tyr Asp Glu Tyr Tyr Asn  
 305 310 315 320  
 Ala Pro Tyr Gly Tyr Asn Asp Lys Ile Trp Val Gly Tyr Asp Asp Leu  
 325 330 335  
 Ala Ser Ile Ser Cys Lys Leu Ala Phe Leu Lys Glu Leu Gly Val Ser  
 340 345 350  
 Gly Val Met Val Trp Ser Leu Glu Asn Asp Asp Phe Lys Gly His Cys  
 355 360 365  
 Gly Pro Lys Asn Pro Leu Leu Asn Lys Val His Asn Met Ile Asn Gly  
 370 375 380  
 Asp Glu Lys Asn Ser Phe Glu Cys Ile Leu Gly Pro Ser Thr Thr Thr  
 385 390 395 400  
 Pro Thr Pro Thr Thr Thr Pro Thr Thr Pro Thr Thr Thr Pro Thr Thr  
 405 410 415  
 Pro Ser Pro Thr Thr Pro Thr Thr Thr Pro Ser Pro Thr Thr Pro Thr  
 420 425 430  
 Thr Thr Pro Ser Pro Thr Thr Pro Thr Thr Thr Pro Ser Pro Thr Thr  
 435 440 445  
 Pro Thr Pro Thr Thr Pro Thr Pro Ala Pro Thr Thr Ser Thr Pro Ser  
 450 455 460  
 Pro Thr Thr Thr Glu His Thr Ser Glu Thr Pro Lys Tyr Thr Thr Tyr  
 465 470 475 480  
 Val Asp Gly His Leu Ile Lys Cys Tyr Lys Glu Gly Asp Ile Pro His  
 485 490 495  
 Pro Thr Asn Ile His Lys Tyr Leu Val Cys Glu Phe Val Asn Gly Gly  
 500 505 510

Trp Trp Val His Ile Met Pro Cys Pro Pro Gly Thr Ile Trp Cys Gln  
 515 520 525

Glu Lys Leu Thr Cys Ile Gly Glu  
 530 535

<210> 22

<211> 1608

<212> DNA

<213> Dermatophagoides farinae

<400> 22

ttcgctata caagtcaatt tttcttgaca ccaaatagtg cccggtggac agggcataat 60  
 atgaaccac cagccaccat taacaaattc acagaccaa tatttgtgta tattggttg 120  
 atgtgggata tcaccttctt tgtaacattt gataagatgt ccatcgacat aggttgata 180  
 ttttgggtgt togtttgtgt gttcggctgt ggttggcgaa ggtgtcgatg ttgtaggggc 240  
 tggtgttggt gttgttggtg ttggtgtggt gggagaagga gttgttgcg gtgtggggg 300  
 agaaggggtt gttgtcgggg tggtgggaga aggggttgtt gtcggggtgg tgggagaagg 360  
 agttgttggc gttgtagtcg gggttgtggg tgctcgtcgtt ggagttggtg tcgttgact 420  
 tggacccaaa atgcattcga aagagttctt ttcacgcga ttaatcatat tatgaacttt 480  
 gttcaacaat ggatttttgc gtcgcagtg accttgaaa tcacatttt ccaatgacca 540  
 aaccatgaca ccagaaacgc ctaattcttt caggaaagcc aacttgcag atatactggc 600  
 cagatcatcg taaccgaccc agattttatc attgtaacca tatggagcat tgtaatatc 660  
 atcgtattgg atatgocatt cttctttttg aaacaattga cacaattcta tatatgagag 720  
 gacaccttct tcaccagaaa tgaaacctgg gggcgacatg ctttggctg gatctccaag 780  
 tttgagtttg cttcgatctt caatgctcca agcacggcca tagaatggaa caccattac 840  
 caatttgcct ctgggtggac cattgttcaa ataatagtgc atggtgtagt tgacattgaa 900  
 ctaagtctct aactatcac tttcatctcg tcttttatat aacggagcat ttgtacggta 960

gtccaattttttatatacat aagctcggtc gattttgtct ttacctggtg atactgcagc 1080  
 agtcaaaatcttccatctgg gttcaaaagc gtctttaagt tctctaacca aagccaaata 1140  
 gttttctttttttttttttt ggtaaccdaa togagatcca ggatactccc aatccaaatc 1200  
 tagacctttttttttttttt ttgcacaaaa gtccaaaact gattgtatga attgttgacg 1260  
 atatgttttttttttttttt tatcggaata tttttccgag cttcataacc aaccaccaag 1320  
 tgaaatctttttttttttttt ctggattctt caatcgcaag ttgttgaaac gttcataacc 1380  
 acgtttttttttttttttttt ttttatcctc ttggtaagga tcgaaaactt gaattgtgta 1440  
 tttgtattttttttttttttt tttttttt tttaaccata cattaaatgt gtacacttga atggatcaat 1500  
 atcttcgatactttttttttt ttttttatg atatacggac catgttccaa cataacaaac 1560  
 aattctctttttttttttttt tttttttt ttatcatt atgatctcgt ttgatgga 1608

<210> 23

<211> 25

<212> PRT

<213> Dermatophagoides farinae

<220>

<223> At location 1, Xaa = any amino acid

<400> 13

Xaa	Leu	Glu	Pro	Lys	Thr	Val	Cys	Tyr	Tyr	Glu	Ser	Trp	Val	His	His
	1								10					15	

Arg	Gln	Gly	Glu	Gly	Lys	Met	Asp	Pro
			20				25	

<210> 24

<211> 33

<212> PRT

<213> Dermatophagoides farinae

<220>

<223> At locations, 18, 28, 31 and 32, Xaa = any amino acid

<400> 24

Ser Ile Lys Arg Asp His Asn Asp Tyr Ser Lys Asn Pro Met Met Ile

1	5	10	15
Val Xaa Tyr Gly Gly Ser Ser Gly Tyr Gln Ser Xaa Lys Arg Xaa Xaa			
20	25	30	

Thr

<210> 21

<211> 31

<212> DNA

<213> Artificial Sequence

<220>

<225> Description of Artificial Sequence: Synthetic  
Primer

<210>

<213> At position 24, n = a, c, t or g

<400> 21

aaacdtgata ataaygatta ytcnaaraay c

31

<210> 26

<211> 31

<212> DNA

<213> Artificial Sequence

<220>

<225> Description of Artificial Sequence: Synthetic  
Primer

<400> 26

aaacdtgata ataaygatta yagyaaraay c

31

<210> 27

<211> 23

<212> DNA

<213> Artificial Sequence

<220>

<225> Description of Artificial Sequence: Synthetic

<223> At locations 12 and 21, n = a, c, t or g

<400> 27

cctttcttcac cnacratcaa ncc

23

<210> 18

<211> 13

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic  
Primer

<220>

<223> At locations 12 and 21, n = a, c, t or g

<400> 28

cctttcttcac cnacratgaa ncc

23

<210> 29

<211> 13

<212> PRT

<213> Dermatophagoides farinae

<400> 29

Gln Tyr Gly Val Thr Gln Ala Val Val Thr Gln Pro Ala

1

5

10

<210> 30

<211> 11

<212> PRT

<213> Dermatophagoides farinae

<400> 30

Asp Glu Leu Leu Met Lys Ser Gly Pro Gly Pro

1

5

10

<210> 31

<211> 24

<212> PRT

<213> Dermatophagoides farinae

<400> 31

Asp Met Glu His Phe Thr Gln His Lys Gly Asn Ala Lys Ala Met Ile  
 1 5 10 15

Ala Val Gly Gly Ser Thr Met Ser  
 20

<210> 32

<211> 21

<212> PRT

<213> Dermatophagoides farinae

<400> 32

Asp Ala Asn Glu Glu Ala Arg Ser Gln Leu Pro Glu Thr Ala Met Val  
 1 5 10 15

Leu Ile Lys Ser Gln  
 20

<210> 33

<211> 21

<212> PRT

<213> Dermatophagoides farinae

<400> 33

Gln Ser Arg Asp Arg Asn Asp Lys Pro Tyr Xaa Ile Val Lys Lys Lys  
 1 5 10 15

Lys Lys Ala Leu Asp  
 20

<210> 34

<211> 1621

<212> DNA

<213> Dermatophagoides farinae

<220>

<221> CDS

<222> (14)..(1540)

<400> 34

agaacttatg aaa atg aaa aga aca ttt gca ttg ttt tgt ata tgg gcc 49  
 Met Lys Thr Thr Phe Ala Leu Phe Cys Ile Trp Ala

Cys	Ile	Gly	Leu	Met	Asn	Ala	Ala	Thr	Lys	Arg	Asp	His	Asn	Asn	Tyr		
	15						20					25					
tgc	aaa	aat	cca	atg	cga	atc	gta	tgt	tat	gtt	gga	aca	tggtcc	gtt		145	
Ser	Lys	Asn	Pro	Met	Arg	Ile	Val	Cys	Tyr	Val	Gly	Thr	Trp	Ser	Val		
	30					35				40							
tat	cat	aaa	gtt	gat	cca	tac	aca	att	gaa	gat	att	gat	cct	ttc	aaa	193	
Tyr	His	Lys	Val	Asp	Pro	Tyr	Thr	Ile	Glu	Asp	Ile	Asp	Pro	Phe	Lys		
	45				50				55			60					
tgt	act	cat	ttg	atg	tat	ggc	ttt	gct	aaa	atc	gat	gaa	tac	aaa	tac	241	
Cys	Thr	His	Leu	Met	Tyr	Gly	Phe	Ala	Lys	Ile	Asp	Glu	Tyr	Lys	Tyr		
			65					70				75					
acc	att	caa	gtt	ttt	gat	cca	ttt	caa	gat	gat	aac	cat	aac	tca	tggt	289	
Thr	Ile	Gln	Val	Phe	Asp	Pro	Phe	Gln	Asp	Asp	Asn	His	Asn	Ser	Trp		
		80					85					90					
gaa	aaa	cac	ggg	tat	gaa	cgt	ttc	aac	aac	ttg	aga	ttg	aag	aat	cca	337	
Glu	Lys	His	Gly	Tyr	Glu	Arg	Phe	Asn	Asn	Leu	Arg	Leu	Lys	Asn	Pro		
	95					100				105							
gaa	ttg	acc	acc	atg	att	tca	ttg	ggc	ggc	tggt	tat	gaa	ggc	tca	gaa	385	
Glu	Leu	Thr	Thr	Met	Ile	Ser	Leu	Gly	Gly	Trp	Tyr	Glu	Gly	Ser	Glu		
	110					115				120							
aaa	tat	tgc	gat	atg	gca	gcc	aat	cca	aca	tat	cgt	cag	caa	ttt	gtt	433	
Lys	Tyr	Ser	Asp	Met	Ala	Ala	Asn	Pro	Thr	Tyr	Arg	Gln	Gln	Phe	Val		
125					130					135				140			
caa	tca	gtt	ttg	gac	ttt	ttg	caa	gaa	tac	aaa	ttc	gat	ggc	cta	gat	481	
Gln	Ser	Val	Leu	Asp	Phe	Leu	Gln	Glu	Tyr	Lys	Phe	Asp	Gly	Leu	Asp		
			145					150					155				
ttg	gat	tggt	gaa	tat	cct	gga	tca	cgg	tta	ggc	aat	cct	aaa	atc	gat	529	
Leu	Asp	Trp	Glu	Tyr	Pro	Gly	Ser	Arg	Leu	Gly	Asn	Pro	Lys	Ile	Asp		
		160					165					170					
aaa	caa	aac	tat	tta	aca	tta	gtt	aga	gaa	ctt	aaa	gag	gca	ttt	gaa	577	
Lys	Gln	Asn	Tyr	Leu	Thr	Leu	Val	Arg	Glu	Leu	Lys	Glu	Ala	Phe	Glu		
	175					180					185						
cct	ttc	ggc	tac	ttg	ttg	act	gcc	gca	gta	tca	ccc	ggc	aaa	gat	aaa	625	
Pro	Phe	Gly	Tyr	Leu	Leu	Thr	Ala	Ala	Val	Ser	Pro	Gly	Lys	Asp	Lys		
	190					195				200							
att	gac	gta	gct	tat	gag	ctc	aaa	gaa	ttg	aac	caa	ttg	ttc	gat	tggt	673	



Ile	Asp	Val	Ala	Tyr	Glu	Leu	Lys	Glu	Leu	Asn	Gln	Leu	Phe	Asp	Trp		
205					210					215					220		
atg	aat	gtc	atg	act	tat	gat	tac	cat	ggc	gga	tgg	gaa	aat	gtt	ttc	721	
Met	Asn	Val	Met	Thr	Tyr	Asp	Tyr	His	Gly	Gly	Trp	Glu	Asn	Val	Phe		
				225					230					235			
ggc	cat	aat	gct	ccg	ttg	tat	aaa	cga	ccc	gat	gaa	acg	gat	gaa	ttg	769	
Gly	His	Asn	Ala	Pro	Leu	Tyr	Lys	Arg	Pro	Asp	Glu	Thr	Asp	Glu	Leu		
			240					245					250				
cac	act	tac	ttc	aat	gtc	aac	tac	acc	atg	cac	tat	tat	ttg	aac	aat	817	
His	Thr	Tyr	Phe	Asn	Val	Asn	Tyr	Thr	Met	His	Tyr	Tyr	Leu	Asn	Asn		
		255					260					265					
ggc	gct	act	cga	gac	aaa	ctr	ggt	atg	ggg	gtt	cca	ttc	tat	ggg	cgt	865	
Gly	Ala	Thr	Arg	Asp	Lys	Leu	Val	Met	Gly	Val	Pro	Phe	Tyr	Gly	Arg		
	270					275					280						
gct	tgg	agc	atc	gaa	gat	cga	agc	aaa	gtc	aaa	ctt	ggc	gat	ccg	gcc	913	
Ala	Trp	Ser	Ile	Glu	Asp	Arg	Ser	Lys	Val	Lys	Leu	Gly	Asp	Pro	Ala		
285					290					295					300		
aaa	ggc	atg	tct	cct	cct	ggt	ttt	att	act	ggt	gaa	gaa	ggg	gtt	ctc	961	
Lys	Gly	Met	Ser	Pro	Pro	Gly	Phe	Ile	Thr	Gly	Glu	Glu	Gly	Val	Leu		
				305					310					315			
tca	tac	atc	gaa	ttg	tgt	cag	tta	ttc	cag	aaa	gaa	gaa	tgg	cat	att	1009	
Ser	Tyr	Ile	Glu	Leu	Cys	Gln	Leu	Phe	Gln	Lys	Glu	Glu	Trp	His	Ile		
			320					325					330				
caa	tac	gat	gaa	tat	tac	aat	gct	cca	tac	gga	tat	aat	gat	aaa	atc	1057	
Gln	Tyr	Asp	Glu	Tyr	Tyr	Asn	Ala	Pro	Tyr	Gly	Tyr	Asn	Asp	Lys	Ile		
		335					340					345					
tgg	ggt	ggg	tac	gat	gat	ctg	gct	agt	ata	tca	tgc	aag	ttg	goc	ttt	1105	
Trp	Val	Gly	Tyr	Asp	Asp	Leu	Ala	Ser	Ile	Ser	Cys	Lys	Leu	Ala	Phe		
	350					355					360						
ctc	aaa	gaa	ttg	ggc	gtc	tct	ggc	gtt	atg	ata	tgg	tca	ttg	gaa	aac	1153	
Leu	Lys	Glu	Leu	Gly	Val	Ser	Gly	Val	Met	Ile	Trp	Ser	Leu	Glu	Asn		
365					370					375					380		
gat	gat	ttc	aaa	ggt	cat	tgc	gga	ccg	aaa	tat	cca	ttg	ttg	aac	aaa	1201	
Asp	Asp	Phe	Lys	Gly	His	Cys	Gly	Pro	Lys	Tyr	Pro	Leu	Leu	Asn	Lys		

Val His Asn Met Ile Asn Gly Asp Glu Lys Asn Ser Tyr Glu Cys Leu  
 400 405 410  
 ttg ggc cca agt aca acc aca cca aca acc acc ccg tca act act 1297  
 Leu Gly Pro Ser Thr Thr Thr Pro Thr Pro Thr Thr Pro Ser Thr Thr  
 415 420 425  
 tcg act acc aca cca acg cct acc acc acc gat agc aca agc gaa aca 1345  
 Ser Thr Thr Thr Pro Thr Pro Thr Thr Asp Ser Thr Ser Glu Thr  
 430 435 440  
 cca aaa tac act acg tat att gat gga cat ttg att aaa tgc tat aaa 1393  
 Pro Lys Tyr Thr Thr Tyr Ile Asp Gly His Leu Ile Lys Cys Tyr Lys  
 445 450 455 460  
 caa ggt tat ctt cca cat cca act gat gtt cat aaa tat tta gtt tgt 1441  
 Gln Gly Tyr Leu Pro His Pro Thr Asp Val His Lys Tyr Leu Val Cys  
 465 470 475  
 gaa tat att gcc aca cca aac ggt ggt tgg tgg gta cac att atg gat 1489  
 Glu Tyr Ile Ala Thr Pro Asn Gly Gly Trp Trp Val His Ile Met Asp  
 480 485 490  
 tgt cca aaa gga act aga tgg cac gca aca tta aaa aat tgt att caa 1537  
 Cys Pro Lys Gly Thr Arg Trp His Ala Thr Leu Lys Asn Cys Ile Gln  
 495 500 505  
 gaa tgatctgata tatttgtaac tgttttttgc taaatgaaat ttaaataaaaa 1590  
 Glu  
 ttatttgaat ccattaaaaa aaaaaaaaaa a 1621  
  
 <210> 35  
 <211> 509  
 <212> PRT  
 <213> Dermatophagoides farinae  
  
 <400> 35  
 Met Lys Thr Thr Phe Ala Leu Phe Cys Ile Trp Ala Cys Ile Gly Leu  
 1 5 10 15  
 Met Asn Ala Ala Thr Lys Arg Asp His Asn Asn Tyr Ser Lys Asn Pro  
 20 25 30  
 Met Arg Ile Val Cys Tyr Val Gly Thr Trp Ser Val Tyr His Lys Val  
 35 40 45

Asp Pro Tyr Thr Ile Glu Asp Ile Asp Pro Phe Lys Cys Thr His Leu  
50 55 60

Met Tyr Gly Phe Ala Lys Ile Asp Glu Tyr Lys Tyr Thr Ile Gln Val  
65 70 75 80

Phe Asp Pro Phe Gln Asp Asp Asn His Asn Ser Trp Glu Lys His Gly  
85 90 95

Tyr Glu Arg Phe Asn Asn Leu Arg Leu Lys Asn Pro Glu Leu Thr Thr  
100 105 110

Met Ile Ser Leu Gly Gly Trp Tyr Glu Gly Ser Glu Lys Tyr Ser Asp  
115 120 125

Met Ala Ala Asn Pro Thr Tyr Arg Gln Gln Phe Val Gln Ser Val Leu  
130 135 140

Asp Phe Leu Gln Glu Tyr Lys Phe Asp Gly Leu Asp Leu Asp Trp Glu  
145 150 155 160

Tyr Pro Gly Ser Arg Leu Gly Asn Pro Lys Ile Asp Lys Gln Asn Tyr  
165 170 175

Leu Thr Leu Val Arg Glu Leu Lys Glu Ala Phe Glu Pro Phe Gly Tyr  
180 185 190

Leu Leu Thr Ala Ala Val Ser Pro Gly Lys Asp Lys Ile Asp Val Ala  
195 200 205

Tyr Glu Leu Lys Glu Leu Asn Gln Leu Phe Asp Trp Met Asn Val Met  
210 215 220

Thr Tyr Asp Tyr His Gly Gly Trp Glu Asn Val Phe Gly His Asn Ala  
225 230 235 240

Pro Leu Tyr Lys Arg Pro Asp Glu Thr Asp Glu Leu His Thr Tyr Phe  
245 250 255

Asn Val Asn Tyr Thr Met His Tyr Tyr Leu Asn Asn Gly Ala Thr Arg  
260 265 270

Asp Lys Leu Val Met Gly Val Pro Phe Tyr Gly Arg Ala Trp Ser Ile  
275 280 285

Pro Pro Gly Phe Ile Thr Gly Glu Glu Gly Val Leu Ser Tyr Ile Glu  
305 310 315 320

Leu Cys Gln Leu Phe Gln Lys Glu Glu Trp His Ile Gln Tyr Asp Glu  
325 330 335

Tyr Tyr Asn Ala Pro Tyr Gly Tyr Asn Asp Lys Ile Trp Val Gly Tyr  
340 345 350

Asp Asp Leu Ala Ser Ile Ser Cys Lys Leu Ala Phe Leu Lys Glu Leu  
355 360 365

Gly Val Ser Gly Val Met Ile Trp Ser Leu Glu Asn Asp Asp Phe Lys  
370 375 380

Gly His Cys Gly Pro Lys Tyr Pro Leu Leu Asn Lys Val His Asn Met  
385 390 395 400

Ile Asn Gly Asp Glu Lys Asn Ser Tyr Glu Cys Leu Leu Gly Pro Ser  
405 410 415

Thr Thr Thr Pro Thr Pro Thr Thr Pro Ser Thr Thr Ser Thr Thr Thr  
420 425 430

Pro Thr Pro Thr Thr Thr Asp Ser Thr Ser Glu Thr Pro Lys Tyr Thr  
435 440 445

Thr Tyr Ile Asp Gly His Leu Ile Lys Cys Tyr Lys Gln Gly Tyr Leu  
450 455 460

Pro His Pro Thr Asp Val His Lys Tyr Leu Val Cys Glu Tyr Ile Ala  
465 470 475 480

Thr Pro Asn Gly Gly Trp Trp Val His Ile Met Asp Cys Pro Lys Gly  
485 490 495

Thr Arg Trp His Ala Thr Leu Lys Asn Cys Ile Gln Glu  
500 505

<210> 36

<211> 1621

<212> DNA

<213> Dermatophagoides farinae

<400> 36

tttttttttt ttttttaatg gattcaataa attttattta aatttcattt agcaaaaaac 60

agttacacac atctagatc attcttgaat acaatttttt aatgttggt gccatctagt 120  
tccttttttt ttttttttt ttgttaccca ccaaccacgg ttgtgtgtgg caatatattc 180  
aaaaatttt ttttttttt tctcagttgg atgtggaaga taaccttggt tatagcattt 240  
aatcaaattt ttttttttt aggtagagta ttttggtggt tggcttggtc tatcgggtgt 300  
ggtagggttt ttttttttt tcgaagtagt tgacgggggt gttggtgttg gtgtggttgt 360  
acttggggtt ttttttttt cgtaagagtt cttttcatca ccattgatca tattgtgaac 420  
tttgttcatt ttttttttt tttttccga atgacctttg aaatcatcgt tttccaatga 480  
ccatatcata ttttttttt ttttcaattc ttgagaaaag gccaaattgc atgatatact 540  
agccagatct ttttttttt agpatttt atcatttatc ccgtatggag cattgttaata 600  
ttcatcgtat ttttttttt tttttcttt ctggaataac tgacacaatt cgatgtatga 660  
gagaacacct ttttttttt tttttaaaac aggaggagac atgccttttg ccggatcgcc 720  
aagtttgact ttttttttt ttctgatgct ccaagcacga ccatagaatg gaacacccat 780  
aacaagtttg ttttttttt tttcttggt caaataatag tgcattggtg agttgacatt 840  
gaagtaagtg ttttttttt ccgtttcatc gggtcgttta tacaacggag cattatggcc 900  
gaaaacattt ttttttttt catggtaatc ataagtcatg acattcatcc aatcgaacaa 960  
ttggttcaat ttttttttt ctaagctac gtcaatttta tttttaccgg gtgatactgc 1020  
ggcagtcacac aatttttttt aaggttcaaa tgctctttta agttctctaa ctaatgttaa 1080  
atagttttgt ttttttttt taggattccc taacogtgat ccaggatatt cccaatccaa 1140  
atctaggcca ttttttttt attcttccaa aaagtccaaa actgattgaa caaattgctg 1200  
acgatatggt ggtttttttt acattatcca atattttttt caaccttcat accaaccacc 1260  
caatgaaatc atggttgctc attctggatt ctccaatctc aagttgttga aacgttcata 1320  
cccggtgttt ttttttttt tctcgttata atcttgaaat ggttcaaaaa ctggaatggt 1380  
ctattcttat ttttttttt tttttttttt atagatcaaa ccaattacatt tcaaaaggatc 1440

tacgattcgc attggatttt tcgaataatt attgtgatat cgttttagtgg ccgcattcat 1560

caagccaatg caggcccata tacaaaacaa tgcaaatgic gttttcattt tcataagttc 1620

t 1621

<210> 37

<211> 1527

<212> DNA

<213> Dermatophagoides farinae

<220>

<221> CDS

<222> (1)..(1527)

<400> 37

atg aaa acg aca ttt gaa ttg ttt tgt ata tgg gcc tgc att ggc ttg 48  
Met Lys Thr Thr Phe Ala Leu Phe Cys Ile Trp Ala Cys Ile Gly Leu  
1 5 10 15

atg aat gcg gcc act aaa cga gat cac aat aat tat tcg aaa aat cca 96  
Met Asn Ala Ala Thr Lys Arg Asp His Asn Asn Tyr Ser Lys Asn Pro  
20 25 30

atg cga atc gta tgt tat gtt gga aca tgg tcc gtt tat cat aaa gtt 144  
Met Arg Ile Val Cys Tyr Val Gly Thr Trp Ser Val Tyr His Lys Val  
35 40 45

gat cca tac aca att gaa gat att gat cct ttc aaa tgt act cat ttg 192  
Asp Pro Tyr Thr Ile Glu Asp Ile Asp Pro Phe Lys Cys Thr His Leu  
50 55 60

atg tat ggt ttt gct aaa atc gat gaa tac aaa tac acc att caa gtt 240  
Met Tyr Gly Phe Ala Lys Ile Asp Glu Tyr Lys Tyr Thr Ile Gln Val  
65 70 75 80

ttt gat cca ttt caa gat gat aac cat aac tca tgg gaa aaa cac ggg 288  
Phe Asp Pro Phe Gln Asp Asp Asn His Asn Ser Trp Glu Lys His Gly  
85 90 95

tat gaa cgt ttc aac aac ttg aga ttg aag aat cca gaa ttg acc acc 336  
Tyr Glu Arg Phe Asn Asn Leu Arg Leu Lys Asn Pro Glu Leu Thr Thr  
100 105 110

atg att tca ttg ggt ggt tgg tat gaa ggt tca gaa aaa tat tcg gat 384  
Met Ile Ser Leu Gly Gly Trp Tyr Glu Gly Ser Glu Lys Tyr Ser Asp  
115 120 125

atg gca gcc aat cca aca tat cgt cag caa ttt gtt caa tca gtt ttg	432
Met Ala Ala Asn Pro Thr Tyr Arg Gln Gln Phe Val Gln Ser Val Leu	
130 135 140	
gac ttt ttg caa gaa tac aaa ttc gat ggc cta gat ttg gat tgg gaa	480
Asp Phe Leu Gln Glu Tyr Lys Phe Asp Gly Leu Asp Leu Asp Trp Glu	
145 150 155 160	
tat cct gga tca cgg tta ggc aat cct aaa atc gat aaa caa aac tat	528
Tyr Pro Gly Ser Arg Leu Gly Asn Pro Lys Ile Asp Lys Gln Asn Tyr	
165 170 175	
tta aca tta gtt aga gaa ctt aaa gag gca ttt gaa cct ttc ggc tac	576
Leu Thr Leu Val Arg Glu Leu Lys Glu Ala Phe Glu Pro Phe Gly Tyr	
180 185 190	
ttg ttg act gcc gca gta tca ccc ggt aaa gat aaa att gac gta gct	624
Leu Leu Thr Ala Ala Val Ser Pro Gly Lys Asp Lys Ile Asp Val Ala	
195 200 205	
tat gag ctc aaa gaa ttg aac caa ttg ttc gat tgg atg aat gtc atg	672
Tyr Glu Leu Lys Glu Leu Asn Gln Leu Phe Asp Trp Met Asn Val Met	
210 215 220	
act tat gat tac cat ggc gga tgg gaa aat gtt ttc ggc cat aat gct	720
Thr Tyr Asp Tyr His Gly Gly Trp Glu Asn Val Phe Gly His Asn Ala	
225 230 235 240	
ccg ttg tat aaa cga ccc gat gaa acg gat gaa ttg cac act tac ttc	768
Pro Leu Tyr Lys Arg Pro Asp Glu Thr Asp Glu Leu His Thr Tyr Phe	
245 250 255	
aat gtc aac tac acc atg cac tat tat ttg aac aat ggc gct act cga	816
Asn Val Asn Tyr Thr Met His Tyr Tyr Leu Asn Asn Gly Ala Thr Arg	
260 265 270	
gac aaa ctt gtt atg ggt gtt cca ttc tat ggt cgt gct tgg agc atc	864
Asp Lys Leu Val Met Gly Val Pro Phe Tyr Gly Arg Ala Trp Ser Ile	
275 280 285	
gaa gat cga agc aaa gtc aaa ctt ggc gat ccg gcc aaa ggc atg tct	912
Glu Asp Arg Ser Lys Val Lys Leu Gly Asp Pro Ala Lys Gly Met Ser	
290 295 300	

ttg tat tat tta tta cag aaa gaa gaa tgg cat att caa tac gat gaa 1008  
 Leu Cys Ile Leu Ile Gln Lys Glu Glu Trp His Ile Gln Tyr Asp Glu  
 321 330 335

tat tac tat tta tta tta gga tat aat gat aaa atc tgg gtt ggt tac 1056  
 Tyr Tyr Val Ala Ser Tyr Gly Tyr Asn Asp Lys Ile Trp Val Gly Tyr  
 341 345 350

gat gat tta tat tta ata tca tgc aag ttg gcc ttt ctc aaa gaa ttg 1104  
 Asp Asp Ile Ala Ser Ile Ser Cys Lys Leu Ala Phe Leu Lys Glu Leu  
 351 360 365

ggc gtc tta tat tta tta tta tgg tca ttg gaa aac gat gat ttc aaa 1152  
 Gly Val Ser Ile Ile Ile Trp Ser Leu Glu Asn Asp Asp Phe Lys  
 370 375 380

ggt cat tgg tta tta tta tta cca ttg ttg aac aaa gtt cac aat atg 1200  
 Gly His Cys Ile Ile Ile Tyr Pro Leu Leu Asn Lys Val His Asn Met  
 385 395 400

atc aat ggt tat tta tta tta tct tac gaa tgt ctt ttg ggc cca agt 1248  
 Ile Asn Gly Asn Ile Ile Ile Ser Tyr Glu Cys Leu Leu Gly Pro Ser  
 411 410 415

aca acc aca cca tta tta acc acc ccg tca act act tgg act acc aca 1296  
 Thr Thr Thr Pro Thr Pro Thr Thr Pro Ser Thr Thr Ser Thr Thr Thr  
 421 425 430

cca acg cct acc tta tta gat agc aca agc gaa aca cca aaa tac act 1344  
 Pro Thr Pro Thr Thr Thr Asp Ser Thr Ser Glu Thr Pro Lys Tyr Thr  
 435 440 445

acg tat att gat gga cat ttg att aaa tgc tat aaa caa ggt tat ctt 1392  
 Thr Tyr Ile Asp Gly His Leu Ile Lys Cys Tyr Lys Gln Gly Tyr Leu  
 450 455 460

cca cat cca act gat att cat aaa tat tta gtt tgt gaa tat att gcc 1440  
 Pro His Pro Thr Asp Val His Lys Tyr Leu Val Cys Glu Tyr Ile Ala  
 465 470 475 480

aca cca aac ggt ggt tgg tgg gta cac att atg gat tgt cca aaa gga 1488  
 Thr Pro Asn Gly Gly Trp Trp Val His Ile Met Asp Cys Pro Lys Gly  
 485 490 495

act aga tgg cac gca aca tta aaa aat tgt att caa gaa 1527  
 Thr Arg Trp His Ala Thr Leu Lys Asn Cys Ile Gln Glu  
 500 505



4210> 38

4211> 509

4212> 887

4213> Dermatophagoides farinae

4 1 3-

Met Lys Thr Thr Phe Ala Leu Phe Cys Ile Trp Ala Cys Ile Gly Leu  
1 5 10 15

Met Asp Ala Ala Thr Lys Arg Asp His Asn Asn Tyr Ser Lys Asn Pro  
20 25 30

Met Arg Ile Val Cys Tyr Val Gly Thr Trp Ser Val Tyr His Lys Val  
35 40 45

Asp Phe Tyr Thr Ile Glu Asp Ile Asp Pro Phe Lys Cys Thr His Leu  
50 55 60

Met Tyr Gly Phe Ala Lys Ile Asp Glu Tyr Lys Tyr Thr Ile Gln Val  
65 70 75 80

Phe Asp Phe Phe Gln Asp Asp Asn His Asn Ser Trp Glu Lys His Gly  
85 90 95

Tyr Glu Arg Phe Asn Asn Leu Arg Leu Lys Asn Pro Glu Leu Thr Thr  
100 105 110

Met Ile Ser Leu Gly Gly Trp Tyr Glu Gly Ser Glu Lys Tyr Ser Asp  
115 120 125

Met Ala Ala Asn Pro Thr Tyr Arg Gln Gln Phe Val Gln Ser Val Leu  
130 135 140

Asp Phe Leu Gln Glu Tyr Lys Phe Asp Gly Leu Asp Leu Asp Trp Glu  
145 150 155 160

Tyr Pro Gly Ser Arg Leu Gly Asn Pro Lys Ile Asp Lys Gln Asn Tyr  
165 170 175

Leu Thr Leu Val Arg Glu Leu Lys Glu Ala Phe Glu Pro Phe Gly Tyr  
180 185 190

Leu Leu Thr Ala Ala Val Ser Pro Glu Lys Arg Lys Ile Asn Val Ala

210	215	220
Thr Tyr Asp Tyr His Gly Gly Trp Glu Asn Val Phe Gly His Asn Ala		
225	230	235 240
Pro Leu Tyr Lys Arg Pro Asp Glu Thr Asp Glu Leu His Thr Tyr Phe		
	245	250 255
Asn Val Asn Tyr Thr Met His Tyr Tyr Leu Asn Asn Gly Ala Thr Arg		
	260	265 270
Asp Lys Leu Val Met Gly Val Pro Phe Tyr Gly Arg Ala Trp Ser Ile		
	275	280 285
Glu Asp Arg Ser Lys Val Lys Leu Gly Asp Pro Ala Lys Gly Met Ser		
	290	295 300
Pro Pro Gly Phe Ile Thr Gly Glu Glu Gly Val Leu Ser Tyr Ile Glu		
305	310	315 320
Leu Cys Gln Leu Phe Gln Lys Glu Glu Trp His Ile Gln Tyr Asp Glu		
	325	330 335
Tyr Tyr Asn Ala Pro Tyr Gly Tyr Asn Asp Lys Ile Trp Val Gly Tyr		
	340	345 350
Asp Asp Leu Ala Ser Ile Ser Cys Lys Leu Ala Phe Leu Lys Glu Leu		
	355	360 365
Gly Val Ser Gly Val Met Ile Trp Ser Leu Glu Asn Asp Asp Phe Lys		
	370	375 380
Gly His Cys Gly Pro Lys Tyr Pro Leu Leu Asn Lys Val His Asn Met		
385	390	395 400
Ile Asn Gly Asp Glu Lys Asn Ser Tyr Glu Cys Leu Leu Gly Pro Ser		
	405	410 415
Thr Thr Thr Pro Thr Pro Thr Thr Pro Ser Thr Thr Ser Thr Thr Thr		
	420	425 430
Pro Thr Pro Thr Thr Thr Asp Ser Thr Ser Glu Thr Pro Lys Tyr Thr		
	435	440 445
Thr Tyr Ile Asp Gly His Leu Ile Lys Cys Tyr Lys Gln Gly Tyr Leu		
450	455	460
Pro His Pro Thr Asp Val His Lys Tyr Leu Val Cys Glu Tyr Ile Ala		

465	470	475	480
Thr Pro Asn Gly Gly Trp Trp Val His Ile Met Asp Cys Pro Lys Gly			
485	490	495	
Thr Arg Trp His Ala Thr Leu Lys Asn Cys Ile Gln Glu			
500	505		

&lt;210&gt; 39

&lt;211&gt; 1527

&lt;212&gt; DNA

&lt;213&gt; Dermatophagoides farinae

&lt;400&gt; 39

ttcctgaata caattcttta atgttggttg ccaatttagtt ccttttggac aatccataat 60  
 gtgtaccacac caaccaccgt ttggtgtggc aatatattca caaactaaat atttatgaac 120  
 atcagttgga tgtggaagat aaccttgttt atagcattta atcaaattgt catcaatata 180  
 cgtagtgtat ttgtgtgttt cgtttgtgt atcgggtggtg gtaggcgttg gtgtggtagt 240  
 cgaagtagtt gacgggggtgg ttggtgttgg tgtggttga cttggggcca aaagacattc 300  
 gtaagagttc ttttcattac cattgatcat attgtgaact ttgttcaaca atggatattt 360  
 cgggtccgcaa tgacctttga aatcatcgtt ttccaatgac catatcataa cgccagagac 420  
 gcccaattct ttgagaaagg ccaacttgca tgatatacta gccagatcat cgtaaccaac 480  
 ccagatttta tcatttatat cgtatggagc attgtaatat tcatogtatt gaatatgcca 540  
 ttcttctttc tggaataaact gacacaatto gatgtatgag agaacacott cttcaccagt 600  
 aataaaaacca ggaggagaca tgccttttggc cggatcgcca agtttgactt tgccttcgac 660  
 ttgatgttc caagcacgac catagaatgg aacacccata accagtttgt ctgggttagc 720  
 gccattgttc aaataatagt gcattggtga gttagacattg aagtaagtgt gcaattcatt 780  
 cgtttcatcg ggctgtttat acaacggagc attatggcgg aaaacatttt cccatccgac 840  
 atggtaatca taagtcatga cattcatcga atcgaacaat tggttcaatt ctttgagctc 900

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aggattgact aacogtgatc caggatattc ccaatccaaa tctaggccat cgaatttgta 1080
ttcttcacaa aagtcacaaa ctgattgaac aaattgctga cgatatgttg gattggctgc 1140
catatccgaa tatttttctg aaccttcata ccaaccaccc aatgaaatca tgggtggtcaa 1200
ttctgatttc ttcaatctca agttgttgaa aggttcatac cagtgttttt cccatgagtt 1260
atggttatca tcttgaaatg gatcaaaaac ttgaatgggtg tatttgtatt catcgatttt 1320
aaggaagacc tacatcaaat gagtacattt gaagaggatca atatcttcaa ttgtgtatgg 1380
ttctgatttc tgataaacgg accatgttcc aacataacat acgattcgca ttggattttt 1440
cgaatacttc ttgtgatctc gtttagtggc cgcattcacc aagccaatgc aggcccatat 1500
acaaaacact caaatgtcg ttttcac

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1527

&lt;210&gt; 40

&lt;211&gt; 1470

&lt;212&gt; DNA

&lt;213&gt; Dermatophagoides farinae

&lt;220&gt;

&lt;221&gt; CDS

&lt;222&gt; (1..1470)

&lt;400&gt; 40

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gcc act aaa cga gat cac aat aat tat tcg aaa aat cca atg cga atc 48
Ala Thr Lys Arg Asp His Asn Asn Tyr Ser Lys Asn Pro Met Arg Ile
  1             5             10             15

```

```

gta tgt tat gtt gga aca tgg tcc gtt tat cat aaa gtt gat cca tac 96
Val Cys Tyr Val Gly Thr Trp Ser Val Tyr His Lys Val Asp Pro Tyr
      20             25             30

```

```

aca att gaa gat att gat cct ttc aaa tgt act cat ttg atg tat ggt 144
Thr Ile Glu Asp Ile Asp Pro Phe Lys Cys Thr His Leu Met Tyr Gly
      35             40             45

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```

ttt gct aaa atc gat gaa tac aaa tac acc att caa gtt ttt gat cca 192
Phe Ala Lys Ile Asp Glu Tyr Lys Tyr Thr Ile Gln Val Phe Asp Pro
      50             55             60

```

```

ttt caa gat gat aac cat aac tca tgg gaa aaa cac ggg tat gaa cgt 240
Phe Gln Asp Asp Asn His Asn Ser Trp Glu Lys His Gly Tyr Glu Arg

```

65	70	75	80	
ttc aac aac ttg aga ttg aag aat cca gaa ttg acc acc atg att tca				288
Phe Asn Asn Leu Arg Leu Lys Asn Pro Glu Leu Thr Thr Met Ile Ser				
85		90	95	
ttg ggt ggt tgg tat gaa ggt tca gaa aaa tat tgg gat atg gca ccc				336
Leu Gly Gly Trp Tyr Glu Gly Ser Glu Lys Tyr Ser Asp Met Ala Ala				
100	105		110	
aat cca aca tat cgt cag caa ttt gtt caa tca gtt ttg gac ttt ttg				384
Asn Pro Thr Tyr Arg Gln Gln Phe Val Gln Ser Val Leu Asp Phe Leu				
115	120		125	
caa gaa tac aaa ttc gat ggc cta gat ttg gat tgg gaa tat cct gga				432
Gln Glu Tyr Lys Phe Asp Gly Leu Asp Leu Asp Trp Glu Tyr Pro Gly				
130	135		140	
tca cgg tta ggc aat cct aaa atc gat aaa caa aac tat tta aca tta				480
Ser Arg Leu Gly Asn Pro Lys Ile Asp Lys Gln Asn Tyr Leu Thr Leu				
145	150	155	160	
gtt aga gaa ctt aaa gag gca ttt gaa cct ttc ggc tac ttg ttg act				528
Val Arg Glu Leu Lys Glu Ala Phe Glu Pro Phe Gly Tyr Leu Leu Thr				
165	170		175	
gcc gca gta tca ccc ggt aaa gat aaa att gac gta gct tat gag ctc				576
Ala Ala Val Ser Pro Gly Lys Asp Lys Ile Asp Val Ala Tyr Glu Leu				
180	185		190	
aaa gaa ttg aac caa ttg ttc gat tgg atg aat gtc atg act tat gat				624
Lys Glu Leu Asn Gln Leu Phe Asp Trp Met Asn Val Met Thr Tyr Asp				
195	200		205	
tac cat ggc gga tgg gaa aat gtt ttc ggc cat aat gct ccg ttg tat				672
Tyr His Gly Gly Trp Glu Asn Val Phe Gly His Asn Ala Pro Leu Tyr				
210	215		220	
aaa cga ccc gat gaa acg gat gaa ttg cac act tac ttc aat gtc aac				720
Lys Arg Pro Asp Glu Thr Asp Glu Leu His Thr Tyr Phe Asn Val Asn				
225	230	235	240	
tac acc atg cac tat tat ttg aac aat ggc ggt act cga gac aaa ctt				768
Tyr Thr Met His Tyr Tyr Leu Asn Asn Gly Ala Thr Arg Asp Lys Leu				
245	250		255	

260	265	270	
agc aaa gtc aaa ctt ggc gat ccg gcc aaa ggc atg tct cct cct ggt			864
Ser Lys Val Lys Leu Gly Asp Pro Ala Lys Gly Met Ser Pro Pro Gly			
275	280	285	
ttt att act ggt gaa gaa ggt gtt ctc tca tac atc gaa ttg tgt cag			912
Phe Ile Thr Gly Glu Glu Gly Val Leu Ser Tyr Ile Glu Leu Cys Gln			
290	295	300	
tta ttc cag aaa gaa gaa tgg cat att caa tac gat gaa tat tac aat			960
Leu Phe Gln Lys Glu Glu Trp His Ile Gln Tyr Asp Glu Tyr Tyr Asn			
305	310	315	320
gct cca tac gga tat aat gat aaa atc tgg gtt ggt tac gat gat ctg			1008
Ala Pro Tyr Gly Tyr Asn Asp Lys Ile Trp Val Gly Tyr Asp Asp Leu			
325	330	335	
gct agt ata tca tgc aag ttg gcc ttt ctc aaa gaa ttg ggc gtc tct			1056
Ala Ser Ile Ser Cys Lys Leu Ala Phe Leu Lys Glu Leu Gly Val Ser			
340	345	350	
ggc gtt atg ata tgg tca ttg gaa aac gat gat ttc aaa ggt cat tgc			1104
Gly Val Met Ile Trp Ser Leu Glu Asn Asp Asp Phe Lys Gly His Cys			
355	360	365	
gga ccg aaa tat cca ttg ttg aac aaa gtt cac aat atg atc aat ggt			1152
Gly Pro Lys Tyr Pro Leu Leu Asn Lys Val His Asn Met Ile Asn Gly			
370	375	380	
gat gaa aag aac tct tac gaa tgt ctt ttg ggc cca agt aca acc aca			1200
Asp Glu Lys Asn Ser Tyr Glu Cys Leu Leu Gly Pro Ser Thr Thr Thr			
385	390	395	400
cca aca cca acc acc ccg tca act act tcg act acc aca cca acg cct			1248
Pro Thr Pro Thr Thr Pro Ser Thr Thr Ser Thr Thr Thr Pro Thr Pro			
405	410	415	
acc acc acc gat agc aca agc gaa aca cca aaa tac act acg tat att			1296
Thr Thr Thr Asp Ser Thr Ser Glu Thr Pro Lys Tyr Thr Thr Tyr Ile			
420	425	430	
gat gga cat ttg att aaa tgc tat aaa caa ggt tat ctt cca cat cca			1344
Asp Gly His Leu Ile Lys Cys Tyr Lys Gln Gly Tyr Leu Pro His Pro			
435	440	445	
act gat gtt cat aaa tat tta gtt tgt gaa tat att gcc aca cca aac			1392
Thr Asp Val His Lys Tyr Leu Val Cys Glu Tyr Ile Ala Thr Pro Asn			

450                      455                      460  
 ggt ggt tgg tgg gta cac att atg gat tgt cca aaa gga act aga tgg    1440  
 Gly Gly Trp Trp Val His Ile Met Asp Cys Pro Lys Gly Thr Arg Trp  
 465                      470                      475                      480  
 cac gca aca tta aaa aat tgt att caa gaa                      1470  
 His Ala Thr Leu Lys Asn Cys Ile Gln Glu  
                          485                      490  
  
 <210> 41  
 <211> 490  
 <212> PRT  
 <213> Dermatophagoides farinae  
  
 <400> 41  
 Ala Thr Lys Arg Asp His Asn Asn Tyr Ser Lys Asn Pro Met Arg Ile  
   1                      5                      10                      15  
 Val Cys Tyr Val Gly Thr Trp Ser Val Tyr His Lys Val Asp Pro Tyr  
                          20                      25                      30  
 Thr Ile Glu Asp Ile Asp Pro Phe Lys Cys Thr His Leu Met Tyr Gly  
                          35                      40                      45  
 Phe Ala Lys Ile Asp Glu Tyr Lys Tyr Thr Ile Gln Val Phe Asp Pro  
                          50                      55                      60  
 Phe Gln Asp Asp Asn His Asn Ser Trp Glu Lys His Gly Tyr Glu Arg  
                          65                      70                      75                      80  
 Phe Asn Asn Leu Arg Leu Lys Asn Pro Glu Leu Thr Thr Met Ile Ser  
                          85                      90                      95  
 Leu Gly Gly Trp Tyr Glu Gly Ser Glu Lys Tyr Ser Asp Met Ala Ala  
                          100                      105                      110  
 Asn Pro Thr Tyr Arg Gln Gln Phe Val Gln Ser Val Leu Asp Phe Leu  
                          115                      120                      125  
 Gln Glu Tyr Lys Phe Asp Gly Leu Asp Leu Asp Trp Glu Tyr Pro Gly  
                          130                      135                      140

	165		170		175
Ala Ala Val Ser Pro Gly Lys Asp Lys Ile Asp Val Ala Tyr Glu Leu					
	180		185		190
Lys Glu Leu Asn Gln Leu Phe Asp Trp Met Asn Val Met Thr Tyr Asp					
	195		200		205
Tyr His Gly Gly Trp Glu Asn Val Phe Gly His Asn Ala Pro Leu Tyr					
	210		215		220
Lys Arg Pro Asp Glu Thr Asp Glu Leu His Thr Tyr Phe Asn Val Asn					
	225		230		235
					240
Tyr Thr Met His Tyr Tyr Leu Asn Asn Gly Ala Thr Arg Asp Lys Leu					
			245		250
					255
Val Met Gly Val Pro Phe Tyr Gly Arg Ala Trp Ser Ile Glu Asp Arg					
			260		265
					270
Ser Lys Val Lys Leu Gly Asp Pro Ala Lys Gly Met Ser Pro Pro Gly					
			275		280
					285
Phe Ile Thr Gly Glu Glu Gly Val Leu Ser Tyr Ile Glu Leu Cys Gln					
			290		295
					300
Leu Phe Gln Lys Glu Glu Trp His Ile Gln Tyr Asp Glu Tyr Tyr Asn					
			305		310
					315
					320
Ala Pro Tyr Gly Tyr Asn Asp Lys Ile Trp Val Gly Tyr Asp Asp Leu					
			325		330
					335
Ala Ser Ile Ser Cys Lys Leu Ala Phe Leu Lys Glu Leu Gly Val Ser					
			340		345
					350
Gly Val Met Ile Trp Ser Leu Glu Asn Asp Asp Phe Lys Gly His Cys					
			355		360
					365
Gly Pro Lys Tyr Pro Leu Leu Asn Lys Val His Asn Met Ile Asn Gly					
			370		375
					380
Asp Glu Lys Asn Ser Tyr Glu Cys Leu Leu Gly Pro Ser Thr Thr Thr					
			385		390
					395
					400
Pro Thr Pro Thr Thr Pro Ser Thr Thr Ser Thr Thr Thr Pro Thr Pro					
			405		410
					415
Thr Thr Thr Asp Ser Thr Ser Glu Thr Pro Lys Tyr Thr Thr Tyr Ile					



420 425 430  
 Asp Gly Val Ile Lys Cys Tyr Lys Gln Gly Tyr Leu Pro His Pro  
 440 445  
 Thr Asp Val Leu Tyr Leu Val Cys Glu Tyr Ile Ala Thr Pro Asn  
 450 455 460  
 Gly Gly Tyr Ile Val His Ile Met Asp Cys Pro Lys Gly Thr Arg Trp  
 465 470 475 480  
 His Ala Tyr Val Asn Cys Ile Gln Glu  
 490

&lt;210&gt; 42

&lt;211&gt; 1470

&lt;212&gt; DNA

&lt;213&gt; Dermatophagidinae

&lt;400&gt; 42

ttcttgaata ..... ttcttgctg ccactagtt ccttttggac aatccataat 60  
 gtgtaccacac ..... ttcttgctg aatatattca caaactaaat atttatgaac 120  
 atcagttgga ..... ttcttgctg atagcattta atcaaatgtc catcaatata 180  
 cgtagtgtat ..... ttcttgctg atcgggtggtg gtaggcgttg gtgtggtagt 240  
 cgaagtagtt ..... ttcttgctg tctggttgta cttgggacca aaagacatto 300  
 gtaagagttc ..... ttcttgctg attgtgaact ttgttcaaca atggatattt 360  
 cggtcgcgaa ..... ttcttgctg ttccaatgac catatcataa cgccagagac 420  
 gcccaattct ..... ttcttgctg tgatatacta gccagatcat cgttaaccaac 480  
 ccagatttta ..... ttcttgctg attgtaatat tcatcgtatt gaatatgcca 540  
 ttcttcttct ..... ttcttgctg gatgtatgag agaacacatt cttcaccagt 600  
 aataaaaacca ..... ttcttgctg agtttgactt tgcttcgata 660  
 ttgatgtctc ..... ttcttgctg aacacccata acaagtttgt ctgagtagtc 720

atggtaatca taagtcatga cattcatcca atcgaacaat tgggtcaatt ctttgagctc 900  
 ataagctacg tcaattttat ctttaccggg tgatactggg gcagtcaca agtagccgaa 960  
 aggttcaaat ggcctctttaa gttctctaac taatgttaaa tagttttggt tatcgatttt 1020  
 aggattgcct aaccgtgac caggatatto ccaatccaaa tctaggccat cgaatttgta 1080  
 ttcttgcaaa aagtcacaaa ctgattgaac aaattgctga cgatatgttg gattggctgc 1140  
 catatccgaa tattttctg aacctcata ccaaccacc aatgaaatca tgggtggtcaa 1200  
 ttctggatto ttaaatctca agttgttgaa acgttcatac ccgtgttttt cccatgagtt 1260  
 atggttatca tcttgaaatg gatcaaaaaa ttgaatgggtg tatttgtatt catcgatttt 1320  
 agcaaaaacca tacatcaaat gagtacattt gaaaggatca atatcttcaa ttgtgtatgg 1380  
 atcaacttta tgataaacgg accatgttcc aacataacat acgattcgca ttggattttt 1440  
 cgaataatta ttgtgatctc gtttagtggc 1470

<210> 43

<211> 510

<212> DNA

<213> *Dermatophagoides farinae*

<220>

<221> CDS

<222> (1)..(510)

<400> 43

gat atg gaa cat ttt aca caa cat aag ggc aac gcc aaa gcc atg atc 48  
 Asp Met Glu His Phe Thr Gln His Lys Gly Asn Ala Lys Ala Met Ile  
 1 5 10 15

gcc gtc ggt ggt tcg act atg tcc gat caa ttt tcc aag act gca gcg 96  
 Ala Val Gly Gly Ser Thr Met Ser Asp Gln Phe Ser Lys Thr Ala Ala  
 20 25 30

gta gaa cat tat cgg gaa acg ttt gtt gtt agc aca gtt gat ctt atg 144  
 Val Glu His Tyr Arg Glu Thr Phe Val Val Ser Thr Val Asp Leu Met  
 35 40 45

act cgt tat ggt ttc gat ggt gtc atg att gat tgg tct gcc atg caa 192  
 Thr Arg Tyr Gly Phe Asp Gly Val Met Ile Asp Trp Ser Gly Met Gln

50	55	60	
gcc aaa gat agt gat aat ttc att aaa ttg ttg gac aaa ttc gac gaa	240		
Ala Lys Asp Ser Asp Asn Phe Ile Lys Leu Leu Asp Lys Phe Asp Glu			
65	70	75	80
aag ttt gct cac acc tgg ttt gtg atg ggt gtt acc ttg ccg gca acg	288		
Lys Phe Ala His Thr Ser Phe Val Met Gly Val Thr Leu Pro Ala Thr			
85	90	95	
atc gca tca tac gat aac tat aac att cct gcc atc tcc aac tat gtc	336		
Ile Ala Ser Tyr Asp Asn Tyr Asn Ile Pro Ala Ile Ser Asn Tyr Val			
100	105	110	
gat ttt atg aac gtg ctt agt ctg gat tac act gga tca tgg gcc cat	384		
Asp Phe Met Asn Val Leu Ser Leu Asp Tyr Thr Gly Ser Trp Ala His			
115	120	125	
acg gtc ggt cat gct tct ccg ttt cct gaa caa ctc aaa acg cta gaa	432		
Thr Val Gly His Ala Ser Pro Phe Pro Glu Gln Leu Lys Thr Leu Glu			
130	135	140	
gct tac cac aaa cga gcc gct cca cgt cat aag atg gtc atg gct gta	480		
Ala Tyr His Lys Arg Gly Ala Pro Arg His Lys Met Val Met Ala Val			
145	150	155	160
cca ttt tat gca cgt acc tgg att ctc gag	510		
Pro Phe Tyr Ala Arg Thr Trp Ile Leu Glu			
165	170		

&lt;210&gt; 44

&lt;211&gt; 170

&lt;212&gt; PRT

&lt;213&gt; Dermatophagoidea farinae

&lt;400&gt; 44

Asp Met Glu His Phe Thr Gln His Lys Gly Asn Ala Lys Ala Met Ile
1 5 10 15

Ala Val Gly Gly Ser Thr Met Ser Asp Gln Phe Ser Lys Thr Ala Ala
20 25 30

Val Glu His Tyr Arg Glu Thr Phe Val Val Ser Thr Val Asp Leu Met
---

Ala Lys Asp Ser Asp Asn Phe Ile Lys Leu Leu Asp Lys Phe Asp Glu  
65 70 75 80

Lys Phe Ala Val Thr Ser Phe Val Met Gly Val Thr Leu Pro Ala Thr  
85 90 95

Ile Ala Leu Ile Asp Asn Tyr Asn Ile Pro Ala Ile Ser Asn Tyr Val  
100 105 110

Asp Phe Met Val Leu Ser Leu Asp Tyr Thr Gly Ser Trp Ala His  
115 120 125

Thr Val Gly Val Ser Pro Phe Pro Glu Gln Leu Lys Thr Leu Glu  
130 135 140

Ala Tyr His Val Ala Pro Arg His Lys Met Val Met Ala Val  
145 150 155 160

Pro Phe Tyr Ala Ser Thr Trp Ile Leu Glu  
165 170

<210> 45

<211> 510

<212> DNA

<213> Dermatophagoides farinae

<400> 45

ctcgagaatc caggttcgtg cataaaatgg tacagccatg accatcttat gacgtggagc 60  
gcctcgtttg tgataatct ctacggtttt gaggttgtca ggaaacggag aagcatgacc 120  
gaccgtatgg gcccatgata cagtgtaatc cagactaagc acgttcataa aatcgacata 180  
gttggagatg gcaggaatct tatagttatc gtatgatgag atcgttgccg gcaaggtaac 240  
acctatcaca aacgaggtgt gagcaaaactt ttgctcgaat ttgtccaaca atttaatgaa 300  
attatcacta tctttgggtt gcattgccaga ccaatcaatc atgacaccat cgaaaccata 360  
acgagtcata agatcaactg tgctaacaac aaacgtttcc cgataatgtt ctaccgctgc 420  
agtcttggaa aattgacgg acatagtcga accaccgacg gogatcatgg ctttggcggt 480  
gcccttatgt tctgtaaaat gttccatata 510

&lt;213&gt; Artificial Sequence

\*222. Description of Artificial Sequence: Synthetic  
Primer

003 - At location 15, n = a, c t or g

25

©213 - Artificial Sequence

4223 Description of Artificial Sequence: Synthetic  
Primer

17

<213> Artificial Sequence

4223: Description of Artificial Sequence: Synthetic  
Primer

29

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<223> Description of Artificial Sequence: Synthetic  
Primer

<400> 49

gtaatacgac tcactatagg gc

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## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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|---|--|--|---|
| (51) International Patent Classification <sup>6</sup> :<br><b>C07K 14/435, C12N 15/12, A61K<br/>38/17, C07K 16/18, G01N 33/53</b>   |  | <b>A3</b>  | (11) International Publication Number: <b>WO 99/54349</b>       |
|   |  |  | (43) International Publication Date: 28 October 1999 (28.10.99) |
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| (72) Inventors: McCALL, Catherine, Anne; 709 Pleasant Street, Boulder, CO 80302 (US). HUNTER, Shirley, Wu; 2325 Tanglewood Drive, Fort Collins, CO 80525 (US). WEBER, Eric, R.; 2625 Silver Creek Drive, Fort Collins, CO 80525 (US). |  | Published<br><i>With international search report<br/>Before the expiration of the time limit for amending the claims<br/>and to be republished in the event of the receipt of amendments.</i>  |   |
| (74) Agents: CONNELL, Gary, J. et al.; Sheridan Ross P.C., Suite 3500, 1700 Lincoln Street, Denver, CO 80203-4501 (US).   |  | (88) Date of publication of the international search report:<br>3 February 2000 (03.02.00)   |   |
| (54) Title: <b>DERMATOPHAGOIDES NUCLEIC ACID MOLECULES, PROTEINS AND USES THEREOF</b>   |  |  |   |
| (57) Abstract<br><br>The present invention relates to high molecular weight <i>Dermatophagoides</i> proteins, nucleic acid molecules encoding such proteins, and therapeutic and diagnostic reagents derived from such proteins.      |  |  |   |

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| EE | Estonia                  |    |  | SG | Singapore                                    |    |                          |



## INTERNATIONAL SEARCH REPORT

Intern. Application No.

PCT/US 99/08524

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C07K14/435 C12N15/12 A61K38/17 C07K16/18 G01N33/53

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages                              | Relevant to claim No.  |
|------------|---|------------------------|
| A          | EP 0 473 111 A (FUMAKILLA LTD ;UNIV HIROSHIMA (JP)) 4 March 1992 (1992-03-04)<br><br>the whole document         | 1-7,<br>9-22,28,<br>29 |
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|            | ---<br>-/--   |                        |

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

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7 September 1999

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## INTERNATIONAL SEARCH REPORT

Intern: al Application No

PCT/US 99/08524

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages   | Relevant to claim No.  |
|------------|--|------------------------|
| A          | PATENT ABSTRACTS OF JAPAN<br>vol. 095, no. 008,<br>29 September 1995 (1995-09-29)<br>& JP 07 133227 A (FUMAKILLA LTD;OTHERS:<br>01), 23 May 1995 (1995-05-23)<br>abstract  | 1-7,<br>9-22,28,<br>29 |
| A          | ---<br>FUJIKAWA A. ET AL.: "Cloning and<br>characterization of a new allergen, Mag 3,<br>from the house dust mite Dermatophagoides<br>farinae: cross-reactivity with<br>high-molecular-weight allergen."<br>MOLECULAR IMMUNOLOGY,<br>vol. 33, no. 3, 1996, pages 311-319,<br>XP002113314<br>the whole document   | 1-7,<br>9-22,28,<br>29 |
| A          | ---<br>STEWART G.A. ET AL.: "The isolation of an<br>allergen from extracts of Dermatophagoides<br>pteronyssinus using a murine IgA myeloma<br>with a specificity for phosphorylcholine"<br>CLINICAL ALLERGY,<br>vol. 10, no. 5, 1980, pages 617-630,<br>XP002113315<br>the whole document  | 1-7,<br>9-22,28,<br>29 |
| A          | ---<br>STEWART G.A. AND TURNER K.J.:<br>"Physicochemical and immunochemical<br>characterization of the high molecular<br>weight allergens from dermatophagoides<br>pteronyssinus with particular reference to<br>the tridacnin, Con-A and S107 reactive<br>components"<br>AUST. J. EXP. BIOL. MED. SCI.,<br>vol. 58, no. 3, 1980, pages 275-288,<br>XP002113316<br>abstract<br>----- | 1-7,<br>9-22,28,<br>29 |

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US 99/08524

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:  
see FURTHER INFORMATION sheet PCT/ISA/210
2. ☐ Claims Nos.:  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:  
1-7, 9-22, 28, 29 (all searched in part)

Remark on Protest:

☐ The International Search Report was not compiled in compliance with the requirements of Article 17(2)(a).

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.1

Although claims 4 (part b), 5 and 25 are directed to a diagnostic method practised on the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

Although claim 6 is directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

## INTERNATIONAL SEARCH REPORT

Information on patent family members

Intern: International Application No

PCT/US 99/08524

| Patent document<br>cited in search report | Publication<br>date | Patent family<br>member(s) | Publication<br>date |
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